



Variations environnementales et plasticité phénotypique chez le lézard vivipare (*Zootoca vivipara*) : une approche multi-échelle

Alexis Rutschmann

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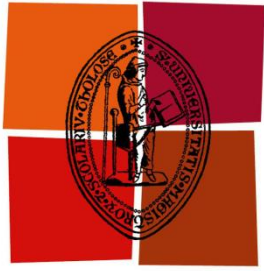
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Université
de Toulouse

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITE DE TOULOUSE

Délivré par : *L'Université Toulouse III - Paul Sabatier*

Discipline ou spécialité : *Ecologie, Biodiversité et Evolution*

Présentée et soutenue par *Alexis Rutschmann*

Le : *07 Avril 2016*

Titre

Réponse plastique du lézard vivipare (*Zootoca vivipare*) aux variations environnementales :
une approche multi-échelle

JURY

Xavier Lambin - Professor - University of Aberdeen (Rapporteur)

Patrice David - Directeur de Recherche - Centre d'Ecologie Fonctionnelle & Evolutive, Montpellier (Rapporteur)

Luis-Miguel Chevin - Chargé de Recherche - Centre d'Ecologie Fonctionnelle & Evolutive, Montpellier (Examineur)

Benoît Pujol - Chargé de Recherche - Evolution & Diversité Biologique, Toulouse (Examineur)

Géraldine Loot - Maître de Conférence - Evolution & Diversité Biologique, Toulouse (Présidente du jury)

Ecole Doctorale : *Sciences Ecologique Vétérinaires Agronomiques et Bioingéniereries*

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"Quand tu arrives en haut de la montagne, continue de grimper"

Proverbe tibétain.

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AVANT PROPOS

Cette thèse de l'école doctorale SEVAB de Toulouse (Sciences Écologiques, Vétérinaires, Agronomiques et Bio-ingénieries) a été réalisée à la Station d'Écologie Théorique et Expérimentale de Moulis (SETE), anciennement Station d'Écologie Expérimentales du CNRS de Moulis (USR 2936). Elle a été financée par une bourse doctorale du ministère de l'Enseignement et de la Recherche. L'ensemble des recherches ont été financé par L'ANR MOBIGEN et par le "laboratoire d'Excellence" TULIP (ANR-10-LABX-41). Cette thèse est constituée d'une introduction générale, d'un résumé des méthodes, de 5 chapitres sous la forme d'articles scientifiques, d'une discussion générale et d'annexes. Le manuscrit de thèse est rédigé en français, mais les articles sont présentés en anglais dans leur version la plus avancée. Sur les 6 articles proposés dans cette thèse, l'un d'entre eux est sous presse dans Journal of Animal Ecology, l'un est soumis à Biology Letters et les autres sont à différents stades de préparation.

INTRODUCTION GENERALE

1. Hétérogénéité environnementale et changements globaux.

L'impact de l'Homme sur la planète est indéniable et au cours des dernières décennies, les activités anthropiques ont modifié les écosystèmes de façon parfois irréversible. L'un des effets les plus médiatiques est certainement celui du réchauffement global de l'atmosphère et des océans, indéniablement accéléré par nos émissions de particules. Ce changement climatique n'est pas homogène sur l'ensemble de la planète mais les différents scénarios existant proposent tout de même une augmentation des températures allant de +0.3°C à +4.8°C, selon les régions et les modèles (IPCC, 2013). Cette augmentation des températures n'a pas pour seule conséquence un réchauffement des milieux mais provoque une cascade d'effets collatéraux. Elle entraîne notamment un changement des régimes de précipitations (assèchement de certaines régions du globe, augmentation des précipitations dans d'autres; Field et al. 2014), une fonte plus importante des glaces, une augmentation du nombre d'évènements climatiques extrêmes (Vasseur et al., 2014) et un dérèglement des principaux courants océaniques.

Ces dérèglements du climat nuisent déjà aux populations animales et végétales. Certains effets sont la conséquence directe du changement climatique : les températures excèdent les limites supportables par les espèces (Sinervo et al., 2010; Huey et al., 2012), le nombre d'incendies augmente (Deutsch et al., 2008) et l'acidification des eaux perturbe le pH des mers et des océans (Foden et al., 2008). D'autres effets, indirects cette fois-ci, sont le résultat des effets cités précédemment : augmentation des pressions de pathogènes, augmentation du taux d'invasions biologiques et modifications des chaînes trophiques (Visser et al., 1998; Dunn et al., 2009; Walther, 2010; Cahill et al., 2012). Ce véritable cocktail d'effets climatiques et de leurs réactions en chaîne, associé à une fragmentation croissante des paysages et une augmentation constante des concentrations de polluants dans les écosystèmes, risque de provoquer l'extinction de 15 à 37% des espèces répertoriées sur la planète d'ici 2050 (Thomas et al., 2004). Face à ce déclin massif des espèces animales et végétales, certains n'hésitent pas à parler de sixième extinction de masse (Wake & Vredenburg, 2008). Voir encadré I pour plus de précisions.

Encadré I: Susceptibilité des espèces au changement globaux

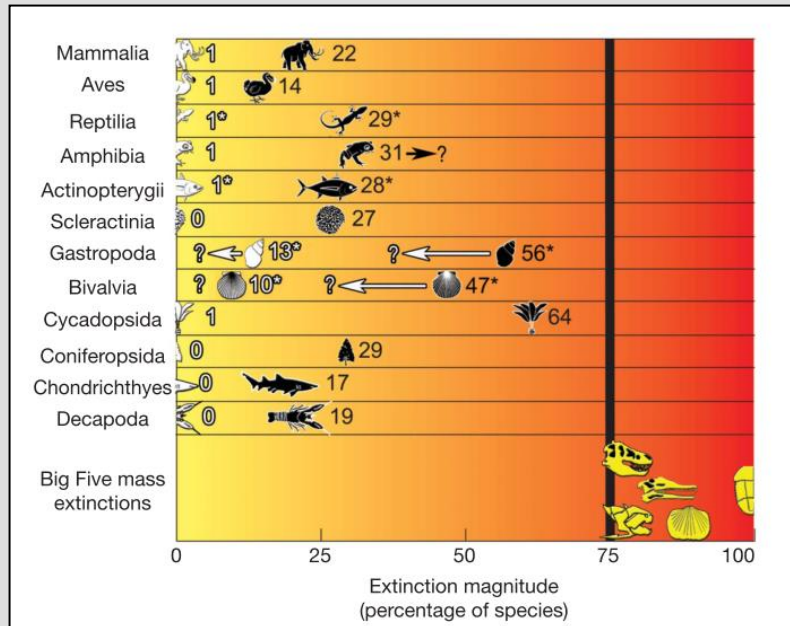


Figure I.1: Projection des pertes de biodiversité animales d'après les listes de l'IUCN (d'après Barnosky et al. 2011). Même si les chiffres varient entre études, la plupart des taxons sont menacés par les changements globaux. En blanc: pourcentage des espèces recensées disparues au cours des 500 dernières années. En noir : pourcentage d'espèces sous statut "menacée" ou "éteinte" d'après l'IUCN. Les flèches blanches indiquent une surestimation potentielle du taux d'extinction (les espèces menacées sont identifiées en priorité). Les flèches noires représentent une sous estimation des risques d'extinction. Les astérisques représentent les taxons pour lesquels la majeure partie des espèces n'ont pas encore été découvertes. En jaune sont représentés les taux d'extinctions des cinq précédentes extinctions de masse (Crétacé, Dévonien, Trias, Ordovicien, Permien).

Les taux d'extinctions des reptiles varient entre 12 et 47 % selon les modèles et les régions du globe (Thomas *et al.*, 2004). Certains auteurs prédisent même que d'ici 2080, 20 % des espèces connues actuellement seront éteintes du fait d'une faible acclimatation à l'augmentation des températures (Sinervo *et al.*, 2010).

2. Réponse des espèces aux variations environnementales

Trois grands mécanismes peuvent permettre aux espèces de faire face à ces risques d'extinction : la dispersion, l'adaptation par microévolution et la plasticité phénotypique. Le changement global offre la triste opportunité d'étudier "en direct" l'efficacité de ces réponses face à une rupture drastique et brutale des conditions du milieu. Elle permet aussi d'en étudier les mécanismes sous-jacents.

Dispersion et changement d'aire de répartition.

Lorsque les conditions du milieu ne permettent plus à une espèce de survivre ou de se reproduire, elle peut tenter de trouver refuge dans un habitat qui lui sera plus favorable. Ainsi, suite au réchauffement du climat, de nombreux changements d'aires de répartition vers des latitudes ou altitudes plus élevées ont pu être observés au cours des dernières décennies (Parmesan *et al.*, 1999; Hughes, 2000; Walther *et al.*, 2002; Parmesan & Yohe, 2003; Penuelas & Boada, 2003; Parmesan, 2006; Rosenzweig *et al.*, 2007). Plusieurs facteurs vont cependant conditionner la possibilité pour une espèce de suivre des conditions qui lui sont favorables. Dans un premier temps, il semble évident que l'espèce doit être capable de disperser suffisamment loin et vite pour survivre, paramètre souvent oublié dans les modèles d'aire de répartition (Domisch *et al.*, 2013). Un certain nombre d'espèces de mammifères et d'oiseaux risquent ainsi de disparaître car leurs capacités de dispersion sont tout simplement trop limitées (Devictor *et al.*, 2008). Ensuite, il est nécessaire que les conditions soient favorables à la survie des individus sur l'ensemble du corridor de dispersion (Thomas *et al.*, 2004; Settele *et al.*, 2014). En effet, des conditions propices à la survie doivent être réunies à la fois lors de l'initiation de la dispersion, lors de la phase d'émigration et lors de la phase d'établissement (Clobert J *et al.*, 2001). Outre les barrières climatiques, des barrières telles que des sommets de montagnes (Penuelas & Boada, 2003) ou un paysage trop fragmenté (McInerny *et al.*, 2007) peuvent aussi empêcher les espèces de poursuivre leur dispersion. Finalement, les changements environnementaux peuvent aussi influencer les capacités de dispersion des individus. En effet, alors que la propension des individus à disperser augmente chez certaines espèces parallèlement à l'augmentation des températures (Rosa *et al.*, 2012), elle peut aussi être atténuée (Bonte *et al.*, 2008; Massot *et al.*, 2008).

Adaptation aux nouvelles conditions climatiques

Les adaptations aux conditions locales permettent aux individus d'optimiser leur survie et leur reproduction en fonction de leur micro-environnement (Kawecki & Ebert, 2004). Ces adaptations touchent tout type de traits et sont largement documentées dans la littérature : adaptations physiologiques (Ayres *et al.*, 1994; Eliason *et al.*, 2011; Weber *et al.*, 2011; McKenzie *et al.*, 2013), morphologiques (Taylor, 1991; Parker *et al.*, 2003; Santamaría *et al.*, 2003), phénologiques (Savolainen *et al.*, 2007; Chuine, 2010), comportementales (Cousyn *et al.*, 2001; Greischar & Koskella, 2007), immunitaires (Schmid-Hempel & Ebert, 2003; Irwin, 2007),... Face aux nouvelles pressions dues au changement climatique, des ajustements de même ordre sont observés pour de nombreuses espèces. Les adaptations locales les plus fréquemment mentionnées dans la littérature sont certainement des ajustements de la phénologie (date de mise bas, initiation de la migration, bourgeonnement) que l'on a pu recenser chez plusieurs centaines d'espèces animales ou végétales (Walther *et al.*, 2002; Root *et al.*, 2003; Charmantier *et al.*, 2008; Robinet & Roques, 2010). Dans une étude récente, Parmesan et Yohe (2003) estiment que 62% des 678 espèces qu'ils ont répertoriées ont avancé leur phénologie suite à l'augmentation des températures au cours des 20 dernières années. Outre les variations de phénologie, des changements peuvent être observés dans la taille des individus (Sheridan & Bickford, 2011), dans leur physiologie (Dillon *et al.*, 2010; Hoffmann *et al.*, 2013), leur coloration (Zeuss *et al.*, 2014), ou leur stratégie d'histoire de vie (Ozgul *et al.*, 2009). L'ensemble de ces adaptations à de nouvelles conditions environnementales peuvent être le résultat de plusieurs mécanismes. Ils peuvent provenir d'une sélection des individus les mieux adaptés (i.e. micro-évolution), d'un apport de gènes pré-adaptés par des migrants ou d'une acclimatation des individus (réponse d'ordre plastique).

L'adaptation par micro-évolution est souvent jugée trop lente face à la rapidité des changements climatiques actuels (Bradshaw *et al.*, 2006). En effet, plusieurs facteurs vont conditionner l'apparition d'une réponse micro-évolutive. Tout d'abord, de la variation génétique héritable doit exister dans la population afin qu'un génotype puisse être sélectionné et transmis à la descendance (Falconer, D. S. & Mackay, 1996). Une telle variation génétique a été documentée chez de nombreuses espèces et pour des traits variés (Merilä and Sheldon 2000; Stirling *et al.* 2002; Berteaux 2004; mais voir aussi Wilson *et al.*

2006). Ensuite, de nombreuses contraintes telles que les corrélations génétiques (Réale *et al.*, 2003a), la sélection antagoniste (Lynch & Walsh, 1998), les contraintes physiologiques (Merila *et al.*, 1997) ou l'apport constant d'allèles non adaptés par un flux de migrant (Lenormand, 2002) vont ralentir l'adaptation aux nouvelles conditions du milieu. Malgré cela, de plus en plus d'adaptations génétiques des populations aux changements climatiques sont rapportées dans la littérature (Réale *et al.*, 2003b; Bradshaw *et al.*, 2006; Gienapp *et al.*, 2008) et l'accumulation de suivis à long terme pourrait bien en révéler de nouvelles. Cette sélection des génotypes le mieux adaptés reste néanmoins un mécanisme intergénérationnel et dans le contexte du changement climatique, il n'est pas dit que les conditions de sélection restent les mêmes d'une génération à l'autre. L'efficacité de la microévolution face au changement climatique semble donc dépendre à la fois de la rapidité du changement environnemental et du temps de génération des espèces.

3. Plasticité phénotypique

La plasticité phénotypique permet aussi aux espèces de réagir mais cette fois-ci dans un intervalle de temps très court (intra-génération, effets plastiques trans-générationnels). Elle pourrait donc jouer un rôle crucial dans le maintien des populations face aux changements environnementaux.

Définition et représentation de la plasticité

Dès 1909, Woltereck propose qu'un phénotype ne représente pas la simple expression du génome mais plutôt le résultat d'une interaction entre génome et environnement (Woltereck, 1909). Dès lors, le phénotype ne peut plus être décrit comme une entité constante et il devient nécessaire de le décrire par une fonction liant les valeurs qu'il peut prendre à l'environnement dans lequel il est exprimé. Cette fonction est appelée norme de réaction. Bradshaw proposera en 1965 une définition plus moderne de la plasticité : c'est le processus qui permet à un génotype de produire différents phénotypes en fonction de l'environnement auquel il est exposé (Bradshaw, 1965; Schlichting & Pigliucci, 1998). Ce mécanisme concerne la plupart des traits phénotypiques, qu'ils soient morphologiques, physiologiques, comportementaux ou phénologiques. On distingue en général deux grands types de plasticité: irréversible ou réversible. La plasticité irréversible, le plus souvent

Encadré II: La norme de réaction : représentation mathématique de la plasticité.

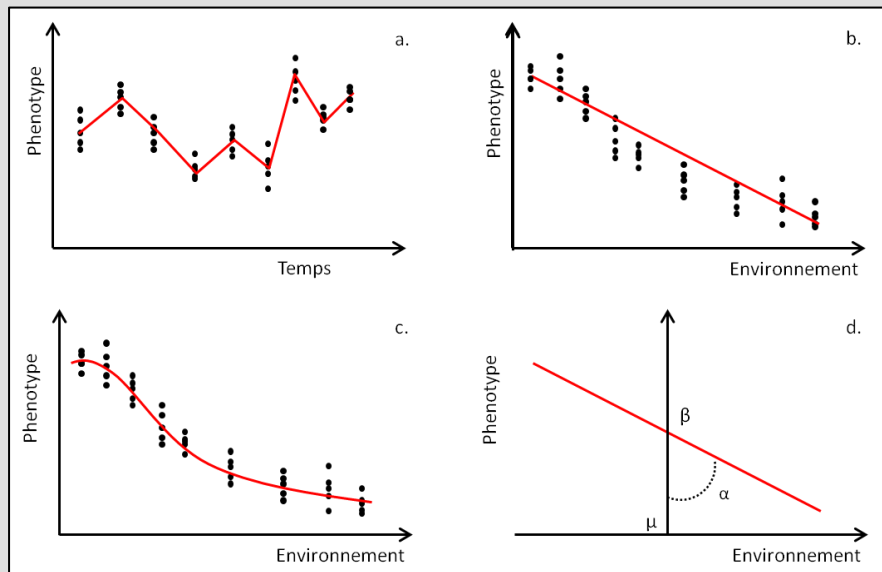


Figure II.1: Représentation mathématique de la plasticité phénotypique. La plasticité phénotypique permet aux individus de répondre aux variations de leur environnement en produisant un phénotype plus adapté à chaque circonstance. (a) Variation phénotypique selon les variations interannuelles de l'environnement. (b) Approximation linéaire de la norme de réaction, fonction reliant chaque niveau du phénotype à l'environnement dans lequel il est exprimé. (c) Représentation polynomiale de la norme de réaction qui est rarement linéaire. (d) Description des paramètres résumant une norme de réaction linéaire de type $\alpha x + \beta$: lorsque les données sont centrées-réduites, β représente le phénotype exprimé par la population dans l'environnement moyen μ qu'elle rencontre. α représente l'ajustement du phénotype pour chaque incrément de l'environnement, autrement dit, l'intensité de la norme de réaction. Cette approche permet notamment de comparer plusieurs normes de réactions entre elles et ainsi de comparer l'intensité de la réponse plastique des individus.

exprimée au cours du développement, fixe la valeur d'un trait pour le reste de la vie de l'individu. Une bonne illustration de ce type de plasticité est le déterminisme sexuel température dépendant chez différentes espèces de crocodiliens ou de testudines (Janzen, 1994). La plasticité réversible, montre au contraire une certaine labilité du phénotype tout au long de la vie de l'individu. Un exemple classique est celui de la variation de la date de ponte des mésanges en fonction des températures du milieu (Visser & Both, 2005; Charmantier *et al.*, 2008).

Classiquement, la réponse plastique est modélisée par sa norme de réaction (voir encadré II et III). Les normes de réactions peuvent prendre plusieurs formes mais en général, elles sont simplifiées au maximum par des représentations linéaires ou polynomiales. L'avantage principal de cette méthode est qu'elle permet d'extraire deux paramètres quantifiables décrivant la norme de réaction. Dans le cas d'une modélisation linéaire, il est possible de caractériser la norme de réaction par son ordonnée à l'origine, décrivant la réponse phénotypique moyenne (si la variable est centrée sur sa moyenne), et sa pente, décrivant l'intensité de la relation (Fig II.1(d) ; Pigliucci 2001). Grâce ces paramètres, il est possible de comparer différentes normes de réactions entre elles, mais aussi de prévoir la réponse d'un individu le long de la gamme d'environnement qu'il rencontre (Nussey *et al.*, 2007; Dingemanse *et al.*, 2009). Il faut cependant garder à l'esprit qu'une norme de réaction ne peut-être linéaire à l'infini et qu'il est compliqué de prévoir sa forme en dehors du cadre de conditions historiquement rencontrées par l'espèce étudiée (Chevin & Lande, 2015).

Plasticité adaptative et évolution de la plasticité

La plasticité phénotypique est considérée comme adaptative lorsqu'elle permet à un individu de répondre à un changement d'environnement en produisant un nouveau phénotype s'approchant du phénotype optimal dans ce nouvel environnement (Pigliucci, 2001). On s'attend à la voir évoluer (*a*) dans des environnements variables, (*b*) si un indice fiable permet de prédire les variations de cet environnement et (*c*) si le phénotype créé dans les différents environnements est avantageux (Gavrilets & Scheiner, 1993; Via *et al.*, 1995). La variation environnementale doit en effet être prédictible sans quoi le phénotype produit ne pourra être en adéquation avec le nouvel environnement et la plasticité sera non-adaptative (Ghalambor *et al.*, 2007a; Reed *et al.*, 2011; Chevin & Lande, 2015). Par ailleurs, le phénotype alternatif produit dans chaque situation environnementale ne doit pas être

Encadré III: Variations des normes de réactions entre populations et individus

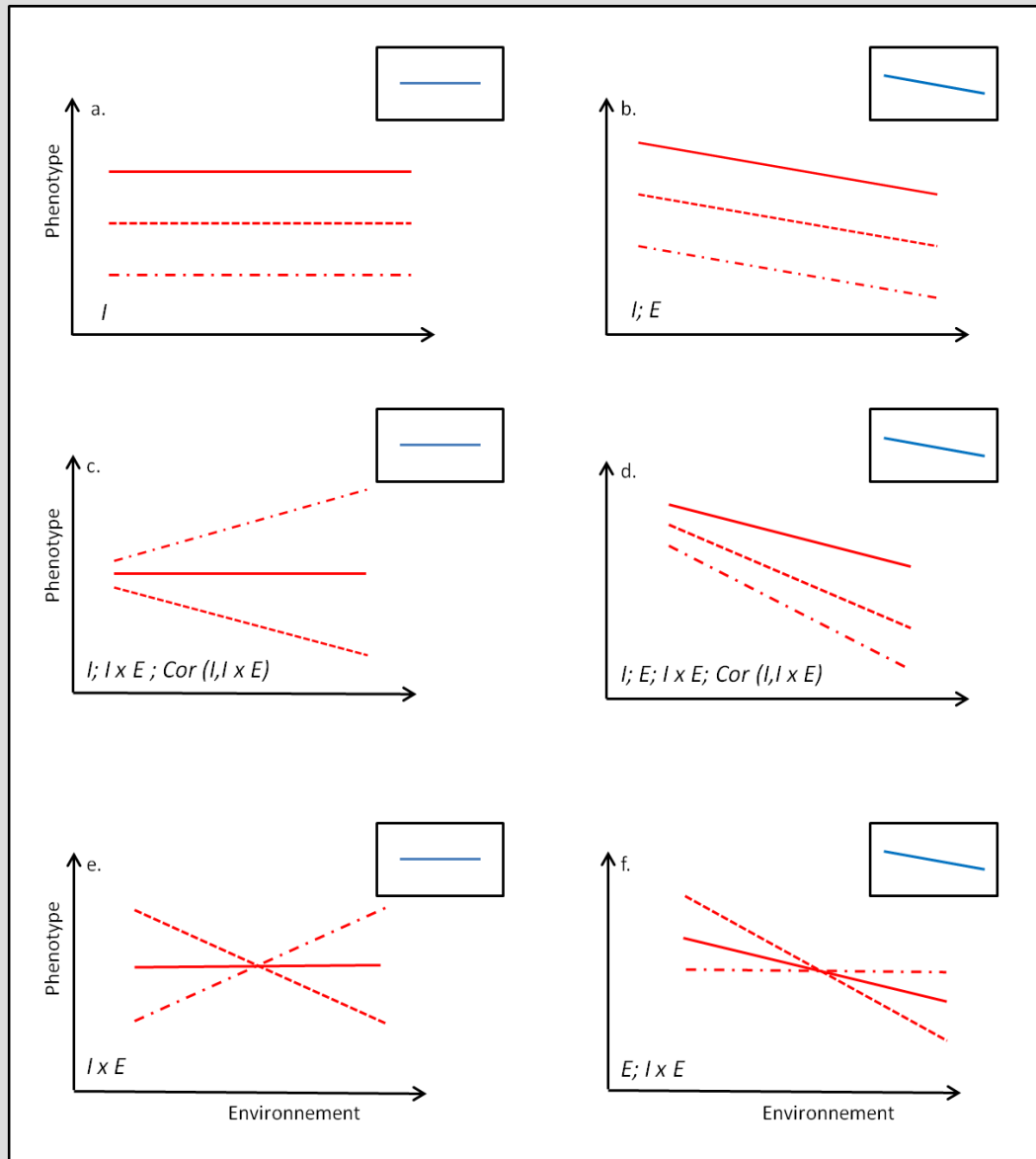


Figure III.1: Différents scénarios de plasticité. Le même raisonnement peut s'effectuer que l'on compare différents individus d'une même population (comme ici) ou différentes populations d'une même espèce. Les lignes rouges représentent les norme d réactions de différents individus de la population et les lignes bleues représentent la plasticité moyenne observée à l'échelle de la population. *I* représente une variation génétique du phénotype moyen entre les individus, *E* représente la présence de plasticité phénotypique détectée à l'échelle de la population, *I* x *E* représente une variation génétique dans l'intensité de la plasticité et *cor(I, I x E)* la corrélation entre le phénotype moyen et l'intensité de la plasticité. D'après Nussey et al., 2007.

surpassé par un phénotype constant à travers tous les environnements. Dans le cas contraire, la compétition entre phénotypes serait remportée par le phénotype canalisé (ou fixé génétiquement) et non pas par le phénotype plastique (Davidson *et al.*, 2011). On pourrait dès lors s'étonner de ne pas rencontrer de réponse plastique absolue, permettant de produire un phénotype "parfait" pour chaque situation de l'environnement. Cette absence suggère que de nombreuses contraintes s'exercent sur la plasticité (Ernande *et al.*, 2004a; Van Buskirk & Steiner, 2009; Murren *et al.*, 2015). Il existe par exemple des coûts de maintenance liés aux systèmes d'acquisition d'information sur l'environnement et ses variations potentielles, indispensable à la réponse plastique (Chevin & Lande, 2015). De la même façon, des coûts sont liés à la production d'un nouveau phénotype dans chaque circonstance environnementale. De plus, différents compromis phénotypiques ou génétiques peuvent restreindre la réponse plastique d'un trait qui, si elle était trop importante, affecterait d'autres caractéristiques de l'individu (Reed). Même si de nombreux travaux théoriques prédisent le rôle essentiel de ces coûts dans l'évolution d'une réponse plastique, peu d'études empiriques ont pu les mettre en évidence à ce jour (Donohue *et al.*, 2000; Agrawal *et al.*, 2002; Murren *et al.*, 2015). Certains auteurs suggèrent que la plasticité serait un tel moteur de l'évolution qu'il en devient difficile d'en détecter les coûts (Chevin & Lande, 2015; Murren *et al.*, 2015).

Variations des normes de réaction : de l'individu à la population

La plasticité phénotypique étant héritable et sous l'emprise de la sélection naturelle, il n'est pas surprenant que les réponses plastiques soient façonnées par les pressions environnementales locales (Nussey *et al.*, 2005a; Pelletier *et al.*, 2007; Charmantier *et al.*, 2008). En effet, nous venons de voir que différents facteurs limitent l'évolution de la plasticité : en fonction des caractéristiques de chaque milieu et des caractéristiques de chaque individu, ils sont susceptibles de contraindre de façon plus ou moins importante les normes de réaction. Un résumé des différents scénarios de plasticité que l'on peut trouver en population naturelle est donné dans l'encadré III. Il est accompagné d'un résumé des méthodes statistiques classiquement utilisées pour différencier ces scénarios (Figure III.2).

Encadré III (suite): Variations des normes de réactions entre populations et individus

Modèle	Structure statistique	Signification biologique
Modèle nul :	$\text{Phénotype} \sim \text{Environnement}$	$p\text{-value}(E) > 0.05$ → Absence de plasticité ² $p\text{-value}(E) < 0.05$ → Présence de plasticité
Effet aléatoire sur l'ordonnée à l'origine (intercept ³):	$\text{Phénotype} \sim \text{Environnement} + \text{aléatoire} = \text{Individus}$	$p\text{-value}(E) > 0.05$ → Absence de plasticité Variation des phénotypes individuels moyens (III.1a) ⁴ $p\text{-value}(E) < 0.05$ → Présence de plasticité Variation des phénotypes individuels moyens (III.1b)
Effet aléatoire sur l'intercepte + effet aléatoire sur la pente + corrélation (intercept/pente) :	$\text{Phénotype} \sim \text{Environnement} + \text{aléatoire} = \text{Individus} + \text{aléatoire} = \text{Individus} * \text{Environnement}$	$p\text{-value}(E) > 0.05$ → Absence de plasticité Variation des phénotypes individuels moyens Variation génétique de la plasticité (III.1c) $p\text{-value}(E) < 0.05$ → Présence de plasticité Variation des phénotypes individuels moyens Variation génétique de la plasticité (III.1d)
Effet aléatoire sur l'intercepte + effet aléatoire sur la pente - corrélation (intercept/pente):	$\text{Phénotype} \sim \text{Environnement} + \text{aléatoire} = \text{Individus} + \text{aléatoire} = \text{Individus} * \text{Environnement}$	$p\text{-value}(E) > 0.05$ → Absence de Plasticité Variation des phénotypes individuels moyens Variation génétique de la plasticité (III.1e) $p\text{-value}(E) < 0.05$ → Présence de plasticité Variation des phénotypes individuels moyens Variation génétique de la plasticité (III.1f)

¹E représente ici l'effet environnemental dans la partie fixe du modèle

²Plasticité détectée (ou non) à l'échelle de la population

³Intercept = Ordonnée à l'origine

⁴Voir Figure III.1 pour une représentation graphique de l'effet

Figure III.2: Différentiation statistique des différents scénarios de plasticité. Des modèles statistiques à effet aléatoires sont en général utilisés pour détecter les différents scénarios de plasticités représentés dans la Figure III.1. Concrètement, il s'agit d'étendre hiérarchiquement la structure d'un modèle de manière à considérer les différents niveaux de variations de la plasticité à l'échelle de la population (astérisque 2; ligne bleue de la Figure III.1) ou des individus (ligne rouge de la Figure III.1). Pour vérifier que chaque effet implémenté augmente significativement la part de variance expliquée, il est possible de comparer les AIC (ou AICc) de chacun des modèles et d'effectuer une sélection de modèle via un « Likelihood Ratio Test » (LRT).

La variation de la réponse plastique existe d'abord à l'échelle individuelle, certains individus répondant de façon plus importante que d'autres aux variations de l'environnement. Plusieurs facteurs influencent les différences interindividuelles telles que la condition physiologique des individus (Nussey *et al.*, 2005a), les conditions environnementales qu'ils rencontrent au cours de leur vie (Nussey *et al.*, 2007) ou leur personnalité (Dingemanse *et al.*, 2009). De nombreuses études s'intéressent à détecter une variation interindividuelle sur des traits plastiques (Husby *et al.*, 2010a; Porlier *et al.*, 2012) et elles ont pu démontrer que celle-ci n'était pas toujours présente. Lorsqu'elle l'est, elle permettra cependant une résilience plus importante de la part de la population à un changement environnemental puisque les normes de réactions les mieux adaptées pourront produire une réponse mieux adaptée aux nouvelles pressions et envahir la population.

Un deuxième niveau de variation se retrouve à l'échelle des populations où la norme de réaction peut être adaptée aux conditions locales. Les travaux empiriques qui s'intéressent aux origines de ces variations entre populations sont encore rares mais quelques études se sont notamment penchées sur la relation entre le niveau d'hétérogénéité environnementale et la forme des normes de réactions. Elles ont pu démontrer expérimentalement (Lind & Johansson, 2007; Baythavong & Stanton, 2010) ou en population naturelle (Nussey *et al.* 2007; Porlier *et al.* 2012, chapitre 1 et 2) que ces variations dans la réponse plastique répondent positivement au niveau d'hétérogénéité de l'environnement et à sa prédictibilité. Elles confirment donc les attendus théoriques. Quel que soit le niveau de variation, comprendre les mécanismes induisant des variations de norme de réaction est crucial pour estimer le potentiel évolutif d'une espèce, et notamment dans le cadre du changement climatique où la plasticité pourrait jouer un rôle clef.

Rôle de la plasticité dans l'évolution

Le rôle crucial que joue la plasticité phénotypique dans l'évolution des populations est connu depuis longtemps (Baldwin, 1896). La plasticité a cependant cela d'ambigu qu'elle peut à la fois être considérée comme un frein et un moteur de la sélection naturelle, et la vitesse d'adaptation à un nouvel environnement va dépendre essentiellement de l'écart entre le phénotype produit par la réponse plastique et l'optimal phénotypique sous les nouvelles

Encadré IV : Plasticité phénotypique: un agent de l'évolution ?

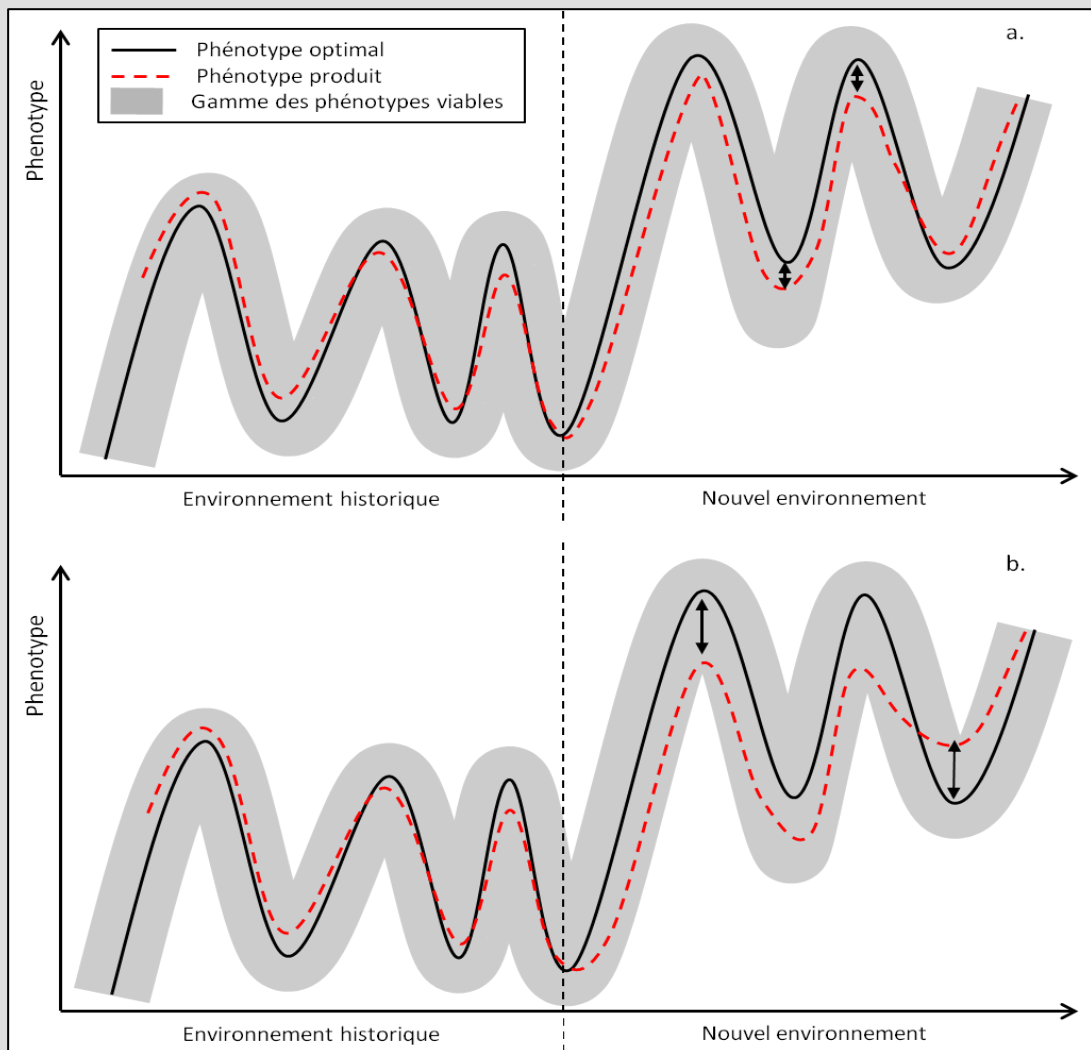


Figure IV.1: Rôle de la réponse plastique dans un nouvel environnement. Le potentiel évolutif apporté par la réponse plastique dans un nouvel environnement va dépendre essentiellement de la qualité du phénotype produit (ligne rouge). (a) Si ce phénotype est proche de l'optimum (ligne noire), il sera rare qu'un nouveau génotype produisant une meilleure réponse apparaisse dans la population et l'envahisse. Dans ce cas, la plasticité peut-être considérée comme un frein à l'évolution puisqu'elle va ralentir la sélection de génome plus adaptés. (b) Si le phénotype est éloignée du phénotype optimal, mais suffisamment proche pour permettre aux individus de survivre et se reproduire dans le nouvel environnement (zone grise), la plasticité pourra être considérée comme un moteur de l'évolution. La plasticité permet en effet aux individus de survivre le temps qu'une sélection sur les génotypes produisant les normes de réactions les mieux adaptées s'opère.

conditions (Figure IV.1). Si elle permet la production de phénotypes proches de l'optimum phénotypique local actuel, les individus pourront être performants, survivre et se reproduire sans qu'une sélection forte pour un nouveau génotype plus performant ne les élimine (Williams *et al.*, 1995; Huey *et al.*, 2003; de Jong, 2005). Elle est dans ce cas un frein à l'adaptation des populations. Au contraire, si les phénotypes produits dans le nouvel environnement sont relativement éloignés de l'optimum local, mais qu'il permet tout de même aux individus de se maintenir dans leur nouvel environnement, on peut considérer la plasticité comme un moteur de l'évolution. En effet, elle va atténuer les effets de la sélection naturelle et permettre aux individus de survivre le temps qu'un phénotype mieux adapté apparaisse dans la population et s'y installe, éliminant la réponse initiale (Pigliucci, 2001; Ghalambor *et al.*, 2007a; Aubret & Shine, 2010; Aubret, 2015). L'évolution des populations peut alors suivre plusieurs scénarios. D'abord, si les normes de réaction varient entre individus (i.e. variation génétique de la norme de réaction, Figure III.1) et que certains génotypes produisent des phénotypes plus adaptés, une sélection directionnelle devrait les privilégier (Badyaev *et al.*, 2005; Reed *et al.*, 2011). Ensuite, si les différences de conditions entre l'environnement d'origine et le nouvel environnement sont importantes, des phénotypes jusque-là cryptiques pourraient alors être exprimés. En effet, au cours de leur évolution les organismes ont développé différents mécanismes permettant d'atténuer l'effet de perturbations génétiques essentiellement dues à des mutations qui s'insèrent dans leur génome. Cette accumulation d'une variation génétique critique pourra s'exprimer dans de nouvelles conditions (Schlichting, 2008). Parmi ces nouveaux phénotypes, la plupart seront neutres ou délétères et rapidement éliminés. Mais si par chance certains augmentent la valeur sélective des individus, ils pourraient au contraire être maintenus et se répandre dans la population (Rutherford, 2000, 2003; Ruden *et al.*, 2003; McGuigan & Sgrò, 2009). Enfin, et bien que ce mécanisme soit encore débattu, si les nouvelles conditions restent stables, une perte de la plasticité se fera au détriment d'un phénotype canalisé, moins coûteux (assimilation génétique; (West-Eberhard, 2003; Schlichting *et al.*, 2006; Lande, 2009; Aubret, 2015).

De plus en plus d'études s'accordent donc pour dire que la plasticité aurait un rôle crucial dans l'établissement de nouvelles populations ou dans la survie des populations face à un

Encadré V : Plasticité adaptative ou non-adaptative: le cas des principales essences forestières européennes.

Il peut s'avérer particulièrement délicat de trancher clairement entre plasticité adaptative et plasticité non adaptative. Un niveau de complexité supérieur intervient notamment chez les espèces ectothermes pour qui tout processus biochimique est directement influencé par la température. Dès lors une réponse plastique peut à la fois refléter une contrainte physiologique et/ou une stratégie adaptative. La température est le premier déterminant de la phénologie pour de nombreuses espèces d'arbres (Chuine, 2010). Avant de pouvoir produire leurs feuilles ou leurs fleurs, les arbres doivent successivement être soumis à une certaine quantité de froid (signe de l'hiver), puis les températures doivent être suffisamment élevées pour être considérées comme un signal de l'arrivée du printemps (Vitasse *et al.*, 2010). Sans ces deux signaux cumulés, le bourgeonnement ne démarrera pas.

Dans une étude récente, Duputié & Rutschmann *et al.* (2015) ont comparé à l'aide d'un modèle mécanistique l'avantage adaptatif d'un phénotype plastique pour le bourgeonnement à un phénotype assimilé (réponse fixe, i.e. dépendant de la photopériode). Ils démontrent que ce mécanisme plastique est adaptatif dans la majeure partie de la distribution : il permet alors, d'une part, d'éviter un gel des jeunes pousses en cas de vague de froid tardive, mais aussi d'augmenter l'assimilation du carbone par les feuilles en cas de printemps précoce. Il permet enfin aux graines, dispersant parfois sur plusieurs dizaines de kilomètres, de produire un adulte capable de survivre et se reproduire dans des conditions parfois très différentes de celle vécues par ses parents. En fonction des espèces la plasticité phénotypique va cependant restreindre la distribution. Pour le hêtre commun (*Fagus sylvatica*) et le chêne pédonculé (*Quercus robur*), elle va limiter l'expansion de l'espèce vers le nord : les périodes de chaleur y étant trop tardives, la feuillaison ne se déclenchera pas suffisamment tôt pour assurer la maturation des fruits avant l'automne. Pour le pin sylvestre au contraire la distribution est contrainte au Sud : il n'existe pas de période de gel et les arbres ne peuvent donc pas sortir de leur période hivernale. L'ensemble de l'article est disponible en Annexe 3.

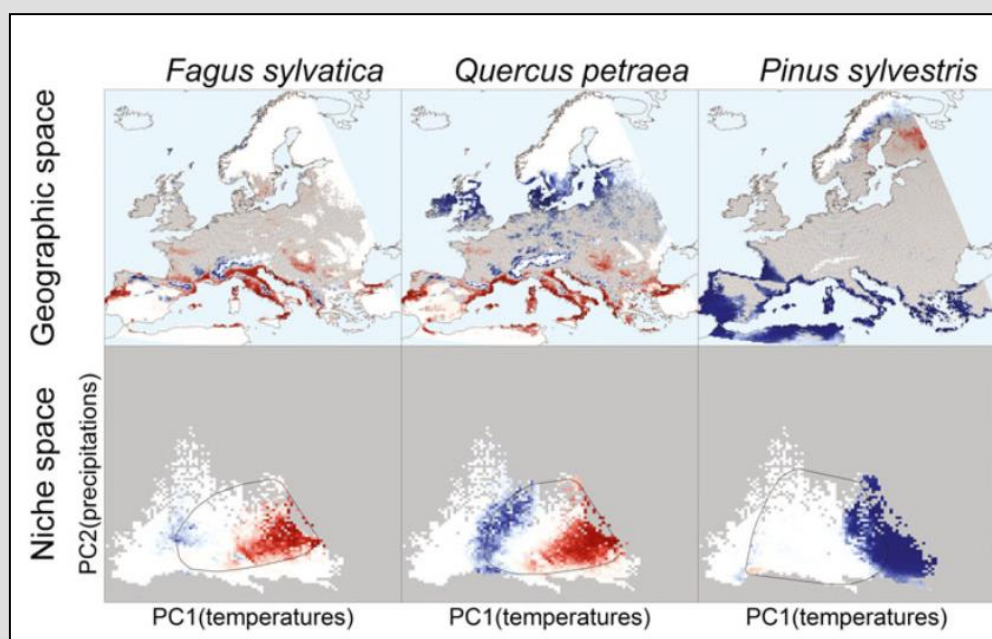


Figure V.1: Contribution de la plasticité à la fitness de trois essences forestières européennes. Les zones bleues indiquent des espaces de la distribution (panels du haut) ou de la niche (panels du bas) où la plasticité est coûteuse, les zones rouges indiquent des zones où la plasticité procure un avantage. La ligne continue noire dans l'espace de la niche indique la niche réalisée de l'espèce.

changement brutal des conditions du milieu (invasion biologique: Richards et al. 2006; Bossdorf et al. 2009; Davidson et al. 2011; colonisation de nouveaux habitats : (Aubret & Shine, 2010; Aubret, 2015), changement climatique : Chevin et al. 2012). Le taux de variation environnementale qu'une population peut endurer, grâce à la plasticité phénotypique, n'est pour autant pas infini. En effet, les normes de réactions sont le fruit d'une sélection sur une gamme précise d'environnements et si le changement environnemental est tel que les nouvelles conditions sortent de cette gamme, il devient très difficile de prédire la forme que prendra la réponse plastique (et donc de prédire si elle sera adaptative ou non). Pour illustrer ce propos nous pouvons prendre l'exemple de la plasticité phénologiques de différentes essences forestière, récemment développé par Morin et al. (2007) et repris par Duputié & Rutschmann et al. (2015). Ces travaux démontrent que si la plasticité est bel et bien adaptative dans la majorité de l'aire de répartition, elle devient extrêmement contraignante dans ses marges et y limite l'expansion des espèces. Voir encadré VI pour plus de précisions.

4. Plasticité phénotypique: le cas des ectothermes

Les amphibiens et les reptiles sont particulièrement sensibles au climat et l'ensemble de leur activité physiologique et métabolique dépend directement de la température du milieu. La plasticité phénotypique est donc présente pour quasiment l'intégralité des traits exprimés chez les ectothermes (morphologie, phénologie, traits d'histoire de vie, comportement,... ; Booth 2006; Urban et al. 2014). Chez ces espèces, il est donc compliqué d'estimer si une réponse plastique reflète une simple contrainte physiologique ou une stratégie adaptative et très peu d'études ont pu mesurer la part adaptative de la plasticité chez des amphibiens ou des reptiles. Urban et al. (2014) ont récemment effectué un recensement de ces études et ont pu montrer que la plasticité était considérée comme adaptative dans 67% des travaux sur les amphibiens et 62% chez les reptiles. Parmi les exemples de plasticité non adaptative, la plupart reposent sur une réponse à des changements extrêmes des températures qui diminuent drastiquement les performances des ectothermes.

Dans le contexte actuel de changement climatique, il devient essentiel de mieux étudier les normes de réactions de ces espèces pour comprendre à quel point elles peuvent être

Encadré VI: Vulnérabilité des ectothermes aux températures.

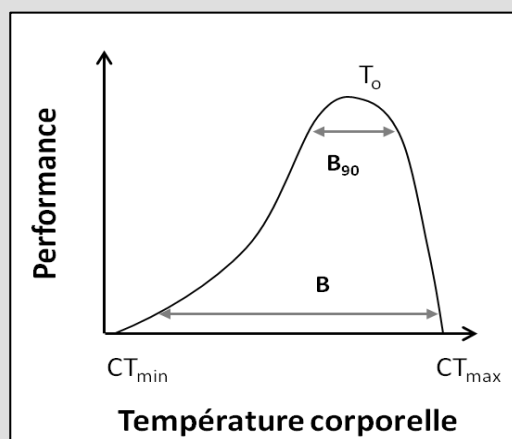


Figure VI.1: Impact de la température corporelle sur les performances d'un ectotherme. La tolérance thermique B s'étend entre les températures critiques minimales et maximales (CT_{min} et CT_{max}) au-delà desquelles aucune performance n'est possible. T_o représente la température corporelle pour laquelle la performance optimale est atteinte. Les individus tendent à maximiser leurs performances restant au delà d'une gamme de température modélisée par B_{90} , zone de températures pour lesquelles la performance est supérieure à 90% de son optimum. D'après Huey & Stevenson 1979.

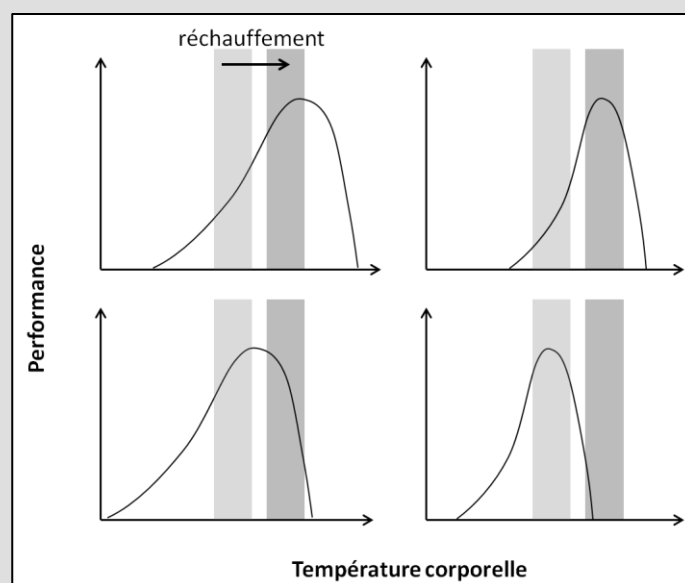


Figure VI.2: Vulnérabilité des ectothermes à une augmentation des températures. L'effet dépendra essentiellement de l'intervalle entre la température actuelle (gamme claire) et la T_o . Des espèces proches de leur optimal thermique (panels du bas) seront plus fragiles face à un réchauffement climatique puisqu'elles basculeront plus facilement au delà de l'optimum de performance. Au contraire, des espèces éloignées de leur T_o verront leur performance améliorées (panels du haut). La vulnérabilité dépendra aussi de la tolérance thermique de l'espèce. De la même façon, des espèces, populations ou individus trop spécialisés (panels de droite) sur une gamme thermique seront plus susceptibles que des espèces pouvant supporter une large gamme thermique (panels de gauche).

vulnérables aux conditions locales de l'environnement. En effet, même si de nombreux facteurs vont déterminer la vulnérabilité d'une espèce face à de tels changements (par exemple la taille de son aire de répartition, sa place dans la chaîne trophique ou sa stratégie générale d'histoire de vie; Dawson et al. 2011; Comte et al. 2014; Conti et al. 2014), l'un des plus important est peut-être la sensibilité physiologique ou métabolique des individus aux conditions climatiques (Dillon *et al.*, 2010; Sinervo *et al.*, 2010).

5. Vulnérabilité des ectothermes au changement climatique

En toute logique, les ectothermes, dont toute activité métabolique dépend des conditions thermiques du milieu, sont considérés comme extrêmement vulnérables à l'augmentation actuelle des températures (IUCN, voir Figure I.1). Leur plasticité face aux variations thermiques de leur environnement est par conséquent l'un des principaux facteurs déterminant leur valeur adaptative (Chuine, 2010).

Courbes thermiques et performance

Il est possible de résumer les performances d'un ectotherme par une courbe en cloche asymétrique que l'on pourrait associer à la norme de réaction de chaque génotype (voir Figure VI.1). Entre les deux températures critiques d'activité (CT_{min} et CT_{max}), les performances vont s'améliorer progressivement avec la température corporelle (T_b) jusqu'à être optimale pour une certaine température (T_o) au-delà de laquelle elles s'effondreront rapidement. Au cours de la journée, la température corporelle des lézards doit donc se stabiliser au plus proche de la température corporelle préférentielle (T_{pref}) afin de maximiser leur performance. Les courbes de performances varient entre individus, populations et espèces. Il est par exemple démontré que leur amplitude est plus large pour les espèces vivant à hautes latitudes ou altitude de par une diminution des CT_{min} dans les environnements plus froids (Hertz *et al.*, 1983; Araújo *et al.*, 2013; Sunday *et al.*, 2014). Une tendance similaire peut s'observer à l'échelle intra-spécifique et des différences de températures à l'échelle locale suffisent à créer de la variance dans les courbes de performances thermiques entre populations (Jonsson *et al.*, 2001; Eliason *et al.*, 2011). L'impact d'une augmentation des températures sur les performances va dépendre essentiellement de la distance entre l'optimal thermique d'une population et la température actuelle (Figure VI.2). En effet, si certaines populations pourraient profiter de cette

augmentation pour s'approcher de leur optimal de performance, d'autres (déjà à l'optimum) pourraient rapidement basculer au delà de leur T_o , ce qui diminuerait fortement leur valeur sélective. Il est intéressant de remarquer que la CT_{max} semble beaucoup plus contrainte que la CT_{min} , rendant un grand nombre d'espèces tropicales vulnérable à une augmentation des températures (Sinervo *et al.*, 2010; Hoffmann *et al.*, 2013).

Maintien des performances physiologiques

Afin de limiter l'impact des variations de températures sur leurs activités, les ectothermes ont développé différentes adaptations leur permettant de maintenir leur température corporelle au plus proche des températures optimales de performance. Parmi elles, les adaptations comportementales jouent un rôle primordial aux deux extrêmes de la gamme thermique. En effet, la thermorégulation permet aux individus d'ajuster leur température corporelle pour augmenter leur activité métabolique. Par exemple, lorsque la température ambiante diminue au cours de la journée (nuage, fin de journée), ils peuvent rejoindre des micro-habitats où la température effective reste élevée (radiation directe ou chaleur rendue par convection, Clusella-Trullas *et al.* 2011). La qualité du micro-habitat est donc primordiale et peut donner lieu à une forte compétition au sein d'espèces territoriales (Huyghe *et al.*, 2005). A l'opposé, de nombreuses espèces tropicales vivent déjà au-delà de leur CT_{max} (Sunday *et al.*, 2014). Aux heures les plus chaudes de la journée, les individus vont adapter leur comportement et se réfugier dans des zones fraîches ou ombragées (Vickers *et al.*, 2011). Ces adaptations comportementales restent cependant limitées. Sinervo *et al.* (2010) ont pu démontrer par exemple qu'avec une augmentation des températures, le temps passé à fuir les températures supérieures à la CT_{max} risque de s'allonger, entraînant une diminution d'activité significative, elle même à l'origine d'une perte de fécondité et de croissance. Par ailleurs, si certaines espèces sont capables d'échapper à ces vagues de chaleurs quotidiennes au sein de leur habitat, d'autres pourraient ne pas avoir cette chance (Huey *et al.*, 2009; Kearney *et al.*, 2009). Par exemple, en zone aride, la probabilité de trouver un refuge frais ou une zone ombragée permettant de maintenir un niveau d'activité convenable est fortement réduite.

6. Principales influences de la température sur le lézard vivipare

Au cours de cette thèse, nous allons nous intéresser essentiellement au lézard vivipare. L'impact des conditions climatiques est au centre des recherches menées sur cette espèce depuis de nombreuses années. L'impact des conditions climatiques sur l'intégralité des traits de cette espèce, la relative facilité d'élevage et de capture et sa répartition au sein de nombreux (mais variés) habitats en font une espèce particulièrement intéressante pour mener des études intégrant différentes échelles écosystémiques (individu, population, écosystème) et plusieurs niveaux de variations spatiales ou temporelles.

Performance et températures

De façon surprenante, il n'existe pas une température optimale unique qui maximise l'ensemble des performances physiologiques du lézard vivipare. Ainsi, celle de la vitesse maximale de course est estimée à 34.2°C, du transit intestinal à 30.2°C et de l'assimilation des nutriments à 33.7°C (Van Damme *et al.*, 1991). Il en résulte un ajustement permanent de la température corporelle (T_b) au cours de la journée en fonction des priorités accordées à chaque trait. Par conséquent, des variations importantes de T_b ont été détectées entre individus (en plus des variations liées au sexe et au statut reproducteur, Artacho *et al.* 2013). La température préférentielle semble quant à elle relativement constante entre populations (T_{pref} moyenne = 31 ± 0.9 °C en Grande Bretagne, T_{pref} moyenne = 32.3 ± 2.5 °C en Belgique, Patterson and Davies 1978; Van Damme *et al.* 1990; Gvozdík and Castilla 2001). Ces résultats peuvent cependant être débattus puisque Bestion *et al.* (*in prep*, Figure VII.2.c) ont démontré expérimentalement l'existence de différences significatives de T_{pref} entre deux traitements climatiques imposés à plusieurs populations de lézards (T_{pref} en conditions froides : 29°C environ, T_{pref} en conditions chaudes : 26.5°C environ). Ces préférences thermiques semblent même transmises à la descendance via un effet maternel. Pour finir, les températures extrêmes que peut supporter le lézard vivipare sont de 2.1°C (± 0.10) pour la borne inférieure et 42°C (± 1.9 °C) pour la limite supérieure (Van Damme *et al.*, 1991; Gvozdík & Castilla, 2001).

Avancement des dates de ponte

La plupart des travaux sur l'influence des conditions environnementales ont été effectuées grâce au suivi continu de populations du Massif Central (Figure IX.1) depuis les années 1980 (dont ROB). Au cours de cette période, des changements climatiques importants ont pu être

Encadré VII: Principales influences du climat sur le lézard vivipare.

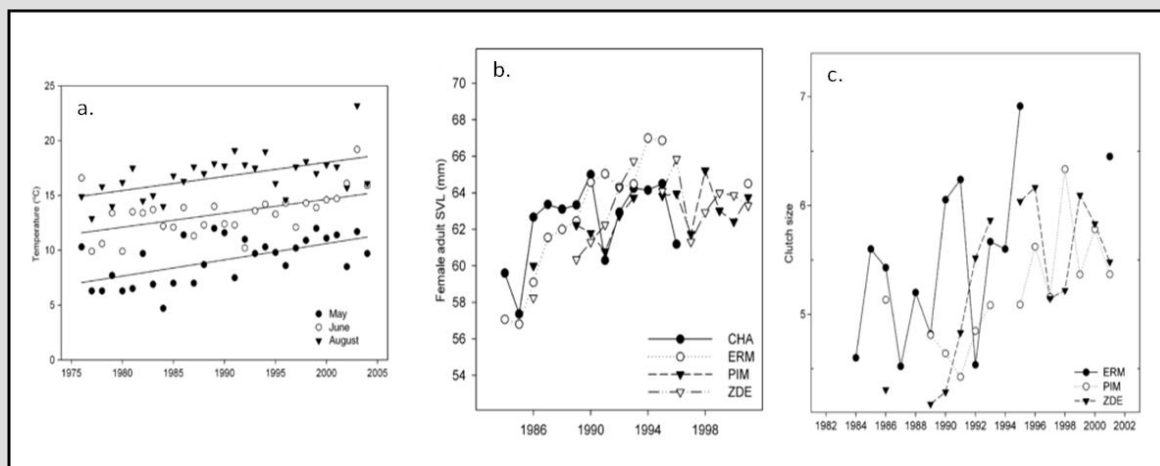


Figure VII.1: Impacte de l'augmentation des températures sur la morphologie des femelles de lézard vivipare. (a) Augmentation des températures de Mai, Juin et Juillet sur le Mont Lozère. (b) Augmentation de la longueur museau-anus (LMA ou SVL pour « snout-vent length », en anglais) sur 4 populations du Mont-Lozère dont ROB (ici baptisée ZDE). (c) Augmentation de la fécondité au cours du temps sur les populations du Mont-Lozère. D'après Chamaillé-Jammes et al., 2006.

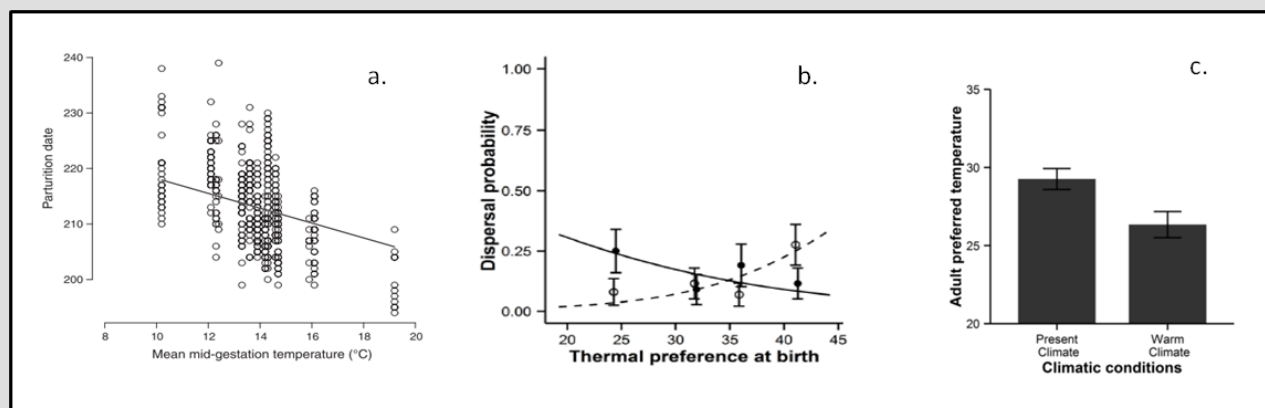


Figure VII.2: Impact des températures sur différents traits du lézard vivipare. (a) Impact de la température à mi-gestation sur la date de mise bas. Les femelles mettent bas plus tôt lorsque les conditions du milieu sont plus chaudes. D'après Le Galliard et al., 2010. (b) Impact des préférences thermiques à la naissance sur la propension des juvéniles à disperser. Le taux de dispersion chez les juvéniles provenant de populations froides (rond blancs) est plus important en conditions chaudes. Le contraire se passe en conditions chaudes. D'après Bestion et al., 2015. (c) Impact des conditions climatiques sur la préférence thermique des individus. Elle est plus élevée en condition froide. D'après Bestion et al. et al., *in prep.*

enregistrés avec notamment une augmentation des moyennes mensuelles des températures journalières des mois de Mai, Juin et Août (Chamaille-Jammes et al. 2006, Figure VII.1). Depuis peu, des résultats expérimentaux, simulant notamment l'effet du changement climatique sont disponibles grâce au Métatron, un dispositif expérimental permettant de mesurer les effets du changement climatique à l'échelle de la population (Legrand *et al.*, 2012).

En réponse à cette augmentation des températures, un avancement important des dates de pontes a pu être observé (Le Galliard *et al.*, 2010). Cet avancement peut avoir plusieurs origines comme un avancement des dates d'émergence et d'accouplement (ce qui est peu probable puisqu'il n'y a pas d'augmentation significative des températures du mois d'Avril, Chamaille-Jammes et al. 2006) ou une accélération du développement embryonnaire (Lorenzon *et al.*, 2001; Bestion *et al.*, 2015). Quoi qu'il en soit, un avancement des dates de pontes peut avoir d'importantes conséquences sur la dynamique d'une population, en permettant notamment l'allongement de la période d'accumulation de réserves précédant l'hibernation.

Taille corporelle

Sur le Mont-Lozère, un accroissement de la taille corporelle des individus a pu être observé. Cette augmentation directement liée à l'augmentation des températures est particulièrement visible chez les sub-adultes (+28%) et les femelles adultes (Chamaille-Jammes *et al.*, 2006). De la même manière, des résultats obtenus dans le Métatron démontrent une croissance annuelle juvénile supérieure dans les conditions les plus chaudes (Bestion *et al.*, 2015). L'accroissement de la taille corporelle juvénile en conditions chaudes est souvent mis en relation avec une accélération des processus biochimiques et physiologiques des individus qui profitent de températures qui tendent vers les températures optimales de performances. Ces deux hypothèses restent cependant relatives à l'intensité de l'augmentation des températures et, si celle-ci est trop importante, un effet négatif des températures est à envisager.

Fécondité

En population naturelle, cet accroissement corporel des femelles adultes entraîne une augmentation de la fécondité, directement liée au volume abdominal des femelles adultes

Encadré VII (suite): Principales influences du climat sur le lézard vivipare.

Table VII.1: Résumé des effets du climat sur différents traits phénotypiques du lézard vivipare. Impact de la température en milieu naturel (principalement sur la population ROB) et dans le Métatron (simulation du climat futur). Les effets de l'humidité sont principalement résumés aux effets des précipitations.

Trait phénotypique	Effet de la température		Effet des précipitations
	Milieu naturel	Climat futur simulé	
Date de Ponte	-	-	NA
Taille corporelle	+	NA	-
Croissance	NA	+	- / +
Fécondité	+	+	+
Probabilité de gravidité	NA	+	NA
Succès reproducteur	-	-	-
Survie (stade)	-	- (adulte)	+ (juvéniles)
Taux de dispersion (juvéniles)	-	Dépendant des T_{pref}	NA

(Chamaille-Jammes *et al.*, 2006). Des résultats similaires sont trouvés dans le Métatron, où la probabilité de gravidité des femelles augmente avec les températures ($p = 0.8 \pm 0.092$ en traitement froid vs $p = 1$ en traitement chaud, Bestion *et al.*, *in prep*). Au contraire, le succès reproducteur (i.e. nombre de juvénile vivants à la naissance) diminue avec l'augmentation des températures, bien que certains individus compensent cet effet par leur comportement de thermorégulation (Bestion *et al.*, 2015).

Survie

En population naturelle, seules les variations interannuelles des températures du mois de mai semblent influencer la survie des adultes (températures conditionnant l'activité en sortie de gestation; Chamaille-Jammes *et al.* 2006). Des expériences visant à estimer l'effet des températures attendues avec le réchauffement climatique ont pu démontrer une diminution de la survie des individus dans les scénarios les plus chauds (Bestion *et al.* 2015, Bestion *et al.*, *in prep*). A une échelle beaucoup plus large, le suivi entre 1980 et 2009 de 46 populations réparties dans toute l'Europe a montré que 30 % des populations se sont éteintes entre 2000 et 2009, une période correspondant à une importante augmentation des températures (Sinervo *et al.*, 2010). Nos prospections personnelles confirment cette tendance puisque depuis 2003, une population semble s'être éteinte (MOT) et deux ont subies une chute d'effectif drastique (CAR, MON, voir Figure IX.1). Cette diminution du taux de survie qui accompagne l'augmentation des températures est très probablement la conséquence directe d'un basculement des performances au delà de leur T_o , entraînant, d'une part, une 'suractivité' des individus pour un rendement moindre et, d'autre part, une diminution des périodes d'inactivité et de repos (Sinervo *et al.*, 2010).

Taux de dispersion

Les études réalisées en populations naturelles ont montré qu'en réponse à l'augmentation des températures de Juin, le nombre de juvéniles dispersant diminuait fortement. Puisque le mois de juin correspond au milieu de la gestation (Massot *et al.*, 2008), il existe donc une influence maternelle sur le taux de dispersion juvénile (Meylan & Clobert, 2004). Expérimentalement, Bestion *et al.* (2015), ont pu démontrer que dans des conditions chaudes, la propension des juvéniles à disperser augmente avec la préférence thermique des individus. Au contraire, dans des conditions plus froides, les individus ont tendance à disperser lorsqu'ils préfèrent des températures plus chaudes. Ces résultats démontrent que

les individus dispersent de façon plus importante lorsque les conditions environnementales ne leur correspondent pas, surlignant l'importance de tenir compte des différences interindividuelles dans les études sur le changement d'aires de répartition.

7. Humidité et lézard vivipare

En plus de la température, l'humidité du milieu est un facteur primordial pour le lézard vivipare. L'humidité relative d'une population est cependant un paramètre plus difficile à mesurer et les études qui se sont concentrées sur ce facteur ont souvent estimé l'impact des précipitations plutôt que celui de l'humidité relative. Elles ont néanmoins pu démontrer des influences diverses de ce facteur sur la dynamique des populations de lézards. Par exemple, une augmentation des précipitations durant la gestation des femelles entraîne une chute du succès reproducteur et une diminution de la taille des juvéniles à la naissance (Lorenzon *et al.*, 1999a; Marquis *et al.*, 2008a; Le Galliard *et al.*, 2010). Au contraire, l'augmentation des précipitations au cours de la vitellogenèse est corrélée positivement au nombre de juvéniles par portée et à une croissance plus importante jusqu'à l'hibernation (Le Galliard *et al.*, 2010). Les précipitations durant le premier mois de vie des juvéniles augmentent quant à elle leur survie tout en diminuant leur croissance (Marquis *et al.*, 2008a). Le rôle de l'humidité du milieu semble complexe et reste largement sous-estimé pour cette espèce vivant majoritairement dans des habitats humides. Dans un contexte de changements dans les régimes des précipitations et d'augmentation des températures, des études complémentaires sont nécessaires, notamment expérimentalement, pour tenter de mesurer l'impact d'un assèchement des populations sur la dynamique des populations de lézards.

QUESTIONS PRINCIPALES ET PLAN DE THESE

La plupart des observations faites en populations naturelles se sont concentrées sur un nombre restreint de populations. Pourtant, il est souvent difficile de généraliser un résultat à partir de si peu de populations (Davis *et al.*, 2005; Pelini *et al.*, 2009). Mon premier objectif a donc été de confirmer sur un ensemble plus large de populations les relations phénotypes-climat historiquement observées par l'équipe. Dans cette optique, 20 populations réparties dans le Massif Central ont été ajoutées en 2003 par l'équipe au suivi des populations historiques (voir Figure IX.1) Au cours de ce travail, je me suis essentiellement intéressé à des traits plastiques, connus pour varier en fonction de l'environnement. De nombreuses études suggèrent en effet que la plasticité phénotypique est un trait héritable et sous sélection, et donc sous l'influence des conditions environnementales locales. Cependant, peu d'études en populations naturelles ont réussi à confirmer les différents attendus théoriques qu'une hétérogénéité environnementale implique. J'ai donc voulu comprendre dans quelles mesures les conditions climatiques locales pouvaient influencer la plasticité phénotypique de la date de mise bas (chapitre 1). De la même façon, je me suis ensuite intéressé à une facette des traits d'histoire de vie plus directement liée à la valeur sélective des individus, à savoir le compromis qui existe entre la taille de ponte et la qualité des juvéniles (chapitre 3).

Dans un second temps, il a été nécessaire de confirmer que les relations observées sur chaque population étaient le fruit de mécanismes individuels, et notamment en ce qui concerne la plasticité de la date de mise bas. Par ailleurs, les relations plastiques sont toujours complexes à interpréter chez les ectothermes, puisque tout processus physiologique est influencé par les conditions thermiques. J'ai donc voulu savoir à quel point les variations de niveau de plasticité entre individus reflétaient une stratégie adaptative ou une contrainte physiologique (chapitre 2).

Mon troisième objectif a été de déterminer si l'ensemble des différences entre populations étaient le reflet d'adaptations physiologiques ou comportementales des lézards à leur environnement. Cet aspect de ma thèse m'a donné l'opportunité d'effectuer plusieurs collaborations axées sur les différences physiologiques des lézards et sur l'impact de

l'humidité du milieu. Ces expériences sont toujours en cours d'analyse et nécessitent parfois des mesures supplémentaires. Je ne présenterai ici que deux aspects de ce travail. D'abord, les résultats obtenus en collaboration avec l'équipe du *CEREP ecotron île-de-France* sur l'acclimatation des individus aux stress hydrique de leur milieu (chapitre A1). Ensuite, le travail effectué avec Qiang Wu, étudiant que j'ai pu encadrer au début de sa thèse. Cette collaboration porte sur les différences de parasitisme entre population et a pu mener à la rédaction d'un article en passe d'être soumis. Ces travaux sont néanmoins placés en annexe puisque je n'en suis pas leur principal auteur (chapitre A2).

Finalement, après m'être attaché à examiner des différences entre populations (chapitres 1, 3 A1 et A2) et entre individus (chapitre 2), je m'intéresse désormais aux mécanismes génétiques pouvant être à l'origine de mes résultats. Pour se faire, j'utilise une approche de génétique quantitative grâce au pédigrée que nous avons pu finir d'assembler au cours de ma thèse. Malheureusement, face à différentes difficultés de mise en place, j'ai manqué de temps pour l'exploiter comme je le désirais. Le pédigrée et ses originalités sont tout de même présentés dans le chapitre 4 de ce manuscrit. Je présente aussi la première analyse utilisant ce pédigrée génétique. Le lézard vivipare est une espèce polygynandrique et les mesures d'héritabilité sont connues pour être affectées par de tels événements de multi-paternités (Charmantier & Reale, 2005). Il m'a donc semblé nécessaire de mesurer cet impact pour une première prise en main du pédigrée (chapitre 5).

MATERIEL ET METHODES

1. Lézard vivipare (*Zootoca vivipara*): biologie et habitat

Le lézard vivipare (*Zootoca vivipara*, Jacquin, précédemment *Lacerta vivipara*) est une espèce largement rependue en Eurasie (voir encadré VIII.1). On retrouve des populations depuis le Nord de l'Espagne jusqu'aux limites du cercle polaire dans les pays Scandinaves. Comme son nom l'indique, cette espèce est vivipare dans toute sa distribution, à l'exception de deux lots de populations ovipares situées dans les Pyrénées et les Alpes du Sud (Surget-groba *et al.*, 2006). Les populations se concentrent en général dans des milieux humides tels que des marais, tourbières, prairies humides ou en bordure de ruisseaux, rivières ou lac, des plaines à l'étage montagnard. Les densités de populations sont en moyenne de 2.7 ± 2.3 individus par m² (Massot *et al.*, 1992)

Ce petit lacertidé mesure entre 50 et 70 mm et pèse à l'âge adulte entre 2 et 5g (hors gestation). La couleur dorsale est en général marron et d'intensité variable en fonctions des individus. Les flancs présentent en générale une ligne noire caractéristique. Les morphotypes dorsaux sont variés, allant d'une ligne noire continue (patron dit 'linéaire') à une réticulation plus complexe (patron dit 'réticulé'). La couleur ventrale varie de façon importante selon le sexe, l'âge et les individus. Les femelles présentent une variation allant du blanc crème à l'orange alors que les mâles sont en général jaunes, oranges et parfois rouges. Les coloration ventrale est absente à la naissance et les dessins dorsaux sont rarement détectables. Ils apparaissent après la première hibernation chez la plus part des individus (voir Figure VIII.2).

Il est possible de diviser les populations de lézards vivipares selon 3 classes d'âges: un stade juvénile, compris entre la naissance de l'individu et sa première hibernation ; un stade sub-adulte rarement reproducteur qui correspond à la première année de vie; et un stade adulte reproducteur. L'espérance de vie des adultes est en moyenne de 4 ans (Size, 1975) mais peut atteindre jusqu'à 7 ans chez certains mâles et 11 ans chez les femelles (Richard *et al.*, 2005). Le cycle de vie est rythmé par une phase d'hibernation obligatoire entre fin Septembre et début Mai. Après une période de vitellogenèse et la fertilisation de leurs œufs, les femelles entament une gestation de deux mois environ. La taille de portée varie entre femelles (entre

Encadré VIII: Distribution et cycle de vie du lézard vivipare.

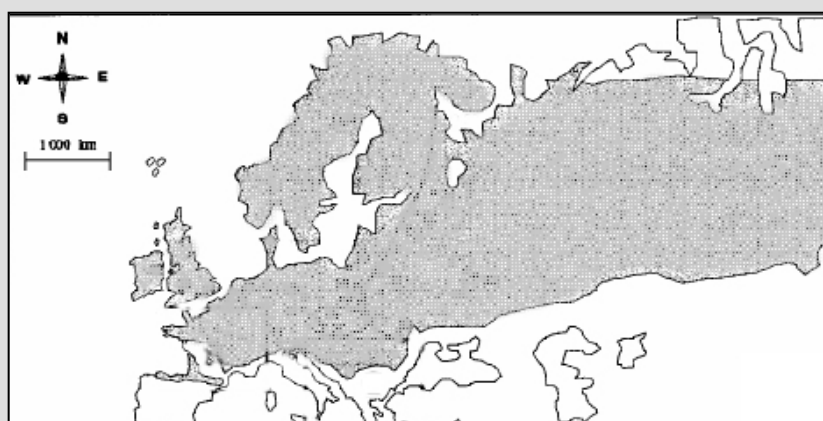


Figure VIII.1: Aire de répartition du lézard vivipare (d'après Surget-Groba et al. 2001, modifié).

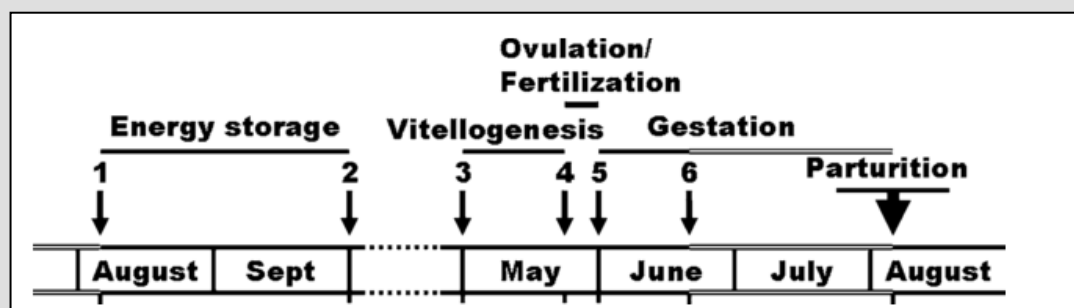


Figure VIII.2: Cycle reproductif type du lézard vivipare sur le Mont-Lozère (modifié de Bleu et al. 2013). Les femelles émergent de leur hibernation début Mai et sont immédiatement fécondée par les mâles ayant émergé quelques jours plus tôt. Une période de vitellogenèse (3) d'un mois précède la fertilisation et la fécondation des œufs(4). La gestation dure environ 2 mois (5). Les lézards sont capturés au premier tiers de la gestation (6) et restent en élevage (ligne blanche) jusqu'à la mise bas (1). S'en suit une période d'accumulation de ressources u début de l'hibernation (2).

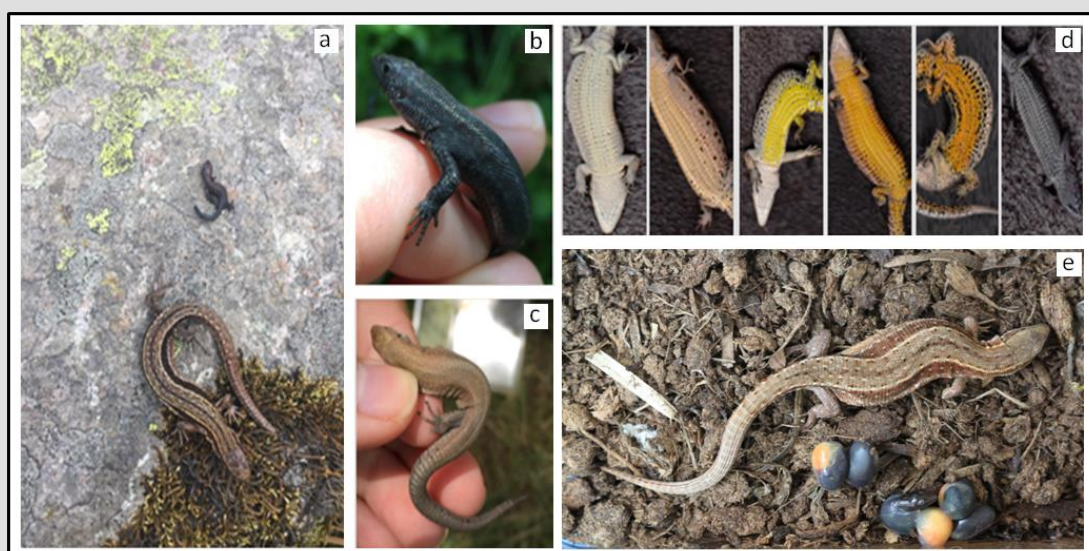


Figure VIII.3 : Lézards vivipares. (a) femelles post-gestantes linéaire avec son juvénile. (b ; c) Cas particuliers de patrons dorsaux (sub-adultes mélanique et monochrome). (d) Couleurs ventrale de plusieurs individus capturés sur le Mont Lozère. (e) Mise bas en laboratoire. Crédits photos: P. Blaimont et A. Rutschmann.

1 et 12 petits) mais est en moyenne de 5 juvéniles. Après la mise bas, elles accumulent des réserves en prévision de la prochaine hibernation (voir Figure VIII.3). On estime qu'environ 25 % des juvéniles survivront à l'hibernation et atteindront le stade sub-adulte. Même si le sex-ratio est équilibré à la naissance, les populations présentent un déséquilibre en ce qui concerne les adultes, avec un sex-ratio biaisé en faveur des femelles (Le Galliard *et al.*, 2005). Il est possible que les mâles, cherchant activement les femelles au moment de la reproduction, s'épuisent rapidement et s'exposent de façon plus importante à la prédation. Chaque individu occupe un territoire bien défini mais de nombreuses interactions sociales existent, notamment entre jeunes individus (Cote & Clobert, 2007).

Active durant la journée, cette espèce chasse de nombreux invertébrés dont des insectes, araignées, gastéropodes, isopodes ou vers (Gonzalez Suarez *et al.*, 2011). Elle-même est la proie d'oiseaux, serpents, amphibiens et d'autres lézards partageant son habitat (notamment au stade juvénile). Les principales techniques de fuite sont le camouflage, la fuite active, le saut et l'autotomie de la queue.

2. *Choix et description des sites d'études*

ROB

La population **Rocher du mas-de-la-Barque** (ROB) est située sur le Mont Lozère dans le Massif Central (44°23'2.72"N, 3°52'40.26"E, altitude: 1421m). Ce site est suivi par capture-marquage-recapture (CMR) tous les ans depuis 1986. Il est composé d'une plaine herbeuse entourée de forêts (bouleaux et sapins principalement) et délimitée à l'Ouest par une rivière. Plusieurs enrochements et des zones de buissons de myrtilles ou de callunes offrent des abris aux lézards. En 2006, le Parc National Naturel des Cévennes a autorisé la construction d'une écurie en amont de la population. Pour se faire, le ruisseau qui traversait la population a été détourné, asséchant la pleine herbeuse principale. Après une diminution de la densité de la population, la dynamique semble avoir retrouvé une certaine stabilité (Massot et Clobert, commentaires personnels).

Global Change

Depuis 2003, le suivi principal a été étendu au suivi de 21 populations réparties sur l'ensemble du Massif Central (projet Global Change, GC). Pour des raisons de place dans le laboratoire et de main d'œuvre, le suivi s'effectue de façon bisannuelle (entre 10 et 12

Encadré IX. Sites d'études du Massif Central

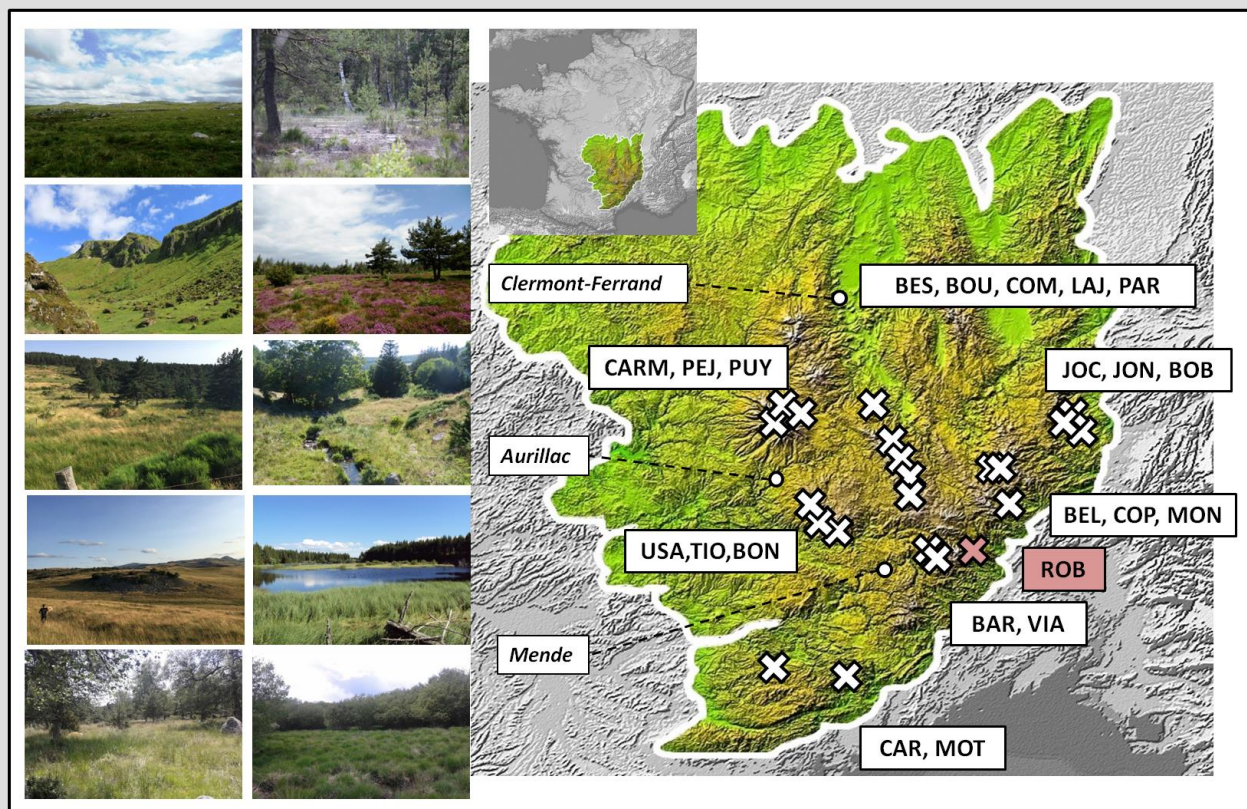


Figure IX.1: Localisation des populations du suivi Global Change et de la population ROB et exemples d'habitats. Les sites de captures sont choisis dans le massif central, au Sud de la répartition de l'espèce. Ils sont répartis selon un double gradient d'anthropisation (forêt ou plaines herbeuses vs pâturage) et de température (plusieurs altitudes et niveaux d'expositions). Illustrations (de haut en bas, de gauche à droite) : TIO, VIA, PUY, MON, COM, JOC, CARM, BAR, BES, CAR.

populations suivies par an). Ces populations ont été initialement choisies parmi 5 massifs et réparties selon un double gradient d'altitude et d'anthropisation dans le but de quantifier d'une part les contraintes thermiques auxquelles peut faire face le lézard vivipare dans son habitat et d'autre part les effets de la transformation d'habitat.

3. Capture, marquage et technique d'élevage

Le suivi de la population ROB est assuré par CMR depuis 1986. Chaque nouvel individu capturé est sexé, pesé, mesuré (longueur museau-anus ou LMA et longueur totale), décrit (couleur, motif dorsal, couleur ventrale, quantité de parasite) puis scanné. La localisation exacte de capture est enregistrée (position GPS) et décrite (type d'habitat). Chaque individu se voit attribuer un numéro unique grâce à une méthode de marquage par '*toe-clipping*'. Cette technique n'affecte ni les capacités locomotrices des individus, ni leur survie ou leur reproduction (Massot *et al.*, 1992). A l'exception des femelles gestantes, les individus capturés sont relâchés à la position exacte de leur capture. Les femelles sont quant à elle apportées au laboratoire où elles sont isolées dans des terrariums individuels (11x18x11cm) au fond recouvert de tourbe. Un abri servant de cache et de zone de thermorégulation est mis à disposition de chaque lézard. Les femelles sont nourries plusieurs fois par semaines avec une teigne ou des grillons et reçoivent de l'eau deux à trois fois par jours. Les lézards ont accès à la lumière du jour et sont chauffés à l'aide d'une ampoule (230V, 25W) 6 heures par jours. Après mise bas, la mère est pesée et chaque juvénile est codé, décrit et sexé. Dans les trois jours suivant la naissance, les individus sont relâchés à la position exacte de capture. Un échantillon de tissus est pris sur chaque individu (phalanges du marquage, extrémité de queue) pour génotypage ultérieur (Richard *et al.*, 2012).

Le même protocole s'applique au suivi GC. Tous les ans entre 20 et 30 femelles sont capturées par population suivie et entrées en élevage. De la même façon, entre 10 et 15 mâles adultes sont capturés pour diverses expériences

4. Données climatiques

Historiquement, les données climatiques de la population **ROB** ont été collectées à la station météorologique du Mont Aigoual, située à environ 50 km à vol d'oiseau. Des stations plus proches sont disponibles, mais aucune ne se situe à une altitude adéquate. Les données

Encadré X. Données récoltées au cours des saisons de terrains

Table X.1 : Résumé de l'ensemble des mesures collectées au cours d'une saison de terrain. Un ensemble de caractéristiques morphologiques, comportementales, génétiques, physiologiques, environnementales ainsi que des performances et des mesures de traits d'histoires de vies sont récoltées chaque année sur les populations du suivi (ROB et/ou GC). Certaines sont collectées en routines depuis le début du projet (indexée 'Routine') alors que d'autres mesures ont été initié pour les besoins de cette thèse et les projets en collaboratifs.

Type de données	Mesures	Populations utilisées	Etude ou collaboration
Morphologiques			
LMA	Longueur Museau-Anus (cm)	Routine (ROB;GC)	/
Masse	Répétées à la capture, en élevage et après parturition (g)	Routine (ROB;GC)	/
Patron dorsaux/ventraux	Scan dorsal et ventral	Routine (ROB;GC)	/
Queue	Etat de la queue (intacte, coupée, régénération)	Routine (ROB;GC)	/
Parasite	Charge parasitaire, type de parasite	Routine (ROB;GC)	Chapitre A2 (Q. Wu)
Traits d'histoire de vie			
Âge	/	Routine (ROB)	/
Fécondité	Nombre de juvéniles vivants, morts, avortés ou mal formés	Routine (ROB; GC)	Chapitre 2
Croissance	Différence de taille entre 2 ans de captures	Routine (ROB)	Chapitre 3
Date de parturition	/	Routine (ROB; GC)	Chapitre 1 et 3
Dispersion	Mesure GPS du point de capture	Routine (ROB)	/
Performances			
Accumulation thermique	Vitesse d'augmentation de la température corporelle	(GC)	Projet en cours (M. Vickers)
Conservation thermique	Vitesse de perte de la température corporelle	(GC)	Projet en cours (M. Vickers)
T pref	Température favorisée par le lézard	(GC)	Projet en cours (D. Miles)
Vitesse de course	Vitesse de course mesurée à plusieurs températures	(GC)	Projet en cours (D. Miles;
Endurance	Temps de course sur un tapis roulant jusqu'à épuisement	(GC)	Projet (D. Miles)
Comportementales			
Agressivité	Comportement face à un objet présenté au lézard : morsure / évitement / absence de réponse	Routine (ROB; GC)	Projet (Q. Wu)
Génétiques			
Echantillon génétique	Identification individuelle et Pédigrée	Routine (ROB)	Chapitre 4 & 5
Echantillon génétique	Différentiation génétique	Routine (GC)	/
Physiologiques			
Osmolalité	Prise de sang et mesure de la concentration d'eau dans le sang	(GC)	Chapitre A1 (A. Dupoué)
Perte hydrique	Perte de masse par évapotranspiration	(GC)	Chapitre A1 (A. Dupoué)
Stress Métabolique	Prise de sang et mesure de la longueur des télomères	(GC)	Projet (A. Dupoué)
Environnementales			
Habitat physique	Substrat à la capture	Routine (ROB;GC)	
Habitat thermique	Température du substrat à la capture	(ROB;GC)	Projet (M. Vickers)
Heure	Heure de capture	(ROB;GC)	Projet (M. Vickers)
Heat Map	Température mesurée en continue dans les différents micro-environnements de capture	(ROB;GC)	Projet (M. Vickers)
Température	Température moyenne, maximale, minimale journalière enregistrée directement sur la population	(ROB;GC)	/
Humidité	Humidité relative enregistrée sur la population	(ROB;GC)	Projet (A. Dupoué)
Anthropisation	Couverture végétale, pression de pâturage	(ROB;GC)	/

récoltées sur le Mont Aigoual nous donnent cependant une bonne estimation des conditions climatiques de **ROB** puisqu'elles sont corrélées aux températures d'une ancienne station météorologique du Mas-de-la-Barque, malheureusement abandonnée depuis les années 1990 ($r = 0.79-0.85$, $n=9-11$, $P < 0.004$ pour les températures Mai, Juin et Août).

Avant ma thèse, les données **GC** n'avaient que très peu utilisées et caractérisées essentiellement par leur localisation (massif, altitude). Cependant, après ma première saison de terrain et les premières analyses, je me suis rendu compte que l'altitude ne suffirait pas à décrire correctement les variations thermiques entre les sites. En effet, les micro-habitats (recouvrement végétal, exposition au vent, ensoleillement, etc.) et la localisation géographique des populations sont à l'origine d'importantes variations climatiques. Malheureusement, aucune mesure des conditions climatiques n'avait été prise *in situ* depuis le début du suivi et chaque population ne pouvait être caractérisée que par les températures des stations météorologiques Météo France voisines. Cependant, certaines populations trop proches les unes des autres ont été décrites par une même station météorologique, parfois éloignée et souvent d'altitude très inférieure (jusqu'à 300m). Pour résoudre ce problème, j'ai placé au cours de l'été 2014 2 à 3 thermocouples par population (iButton®, Waranet Solution) afin de mesurer la température au sol. Les données ont été récoltées sur une plage d'un mois et demi environ (mi-juin à fin juillet). Nous avons utilisé les températures récoltées sur le terrain pour corriger les températures enregistrées par Météo France. L'estimation de la correction a ensuite été appliquée aux périodes en dehors des enregistrements que nous avons réalisés. Malheureusement, seules 14 des 21 populations ont pu être caractérisées courant 2014 et parmi elles seules 11 ont finalement pu être utilisées pour diverses raisons. Nous avons voulu répéter l'opération en 2015, sur l'ensemble des populations cette fois-ci afin pour compléter la base de données. Malheureusement, les températures de juin ont été si élevées que les modèles correctifs ne concordent pas. Il faudra, au cours des prochaines années, trouver un meilleur moyen de caractériser systématiquement la température de chaque population.

5. Caractéristiques individuelles

En plus des données morphologiques mesurées sur le terrain ou en laboratoire, un ensemble de données expérimentales, physiologiques, comportementales ou décrivant

l'habitat de l'individu sont prises en routines sur l'ensemble des populations. D'autres protocoles ont été mis en place pour ce travail de thèse et seront désormais appliqués chaque année (voir encadré X pour un résumé). Les protocoles exacts seront décrits de façon précise dans les chapitres où ils sont utilisés.

CHAPITRE 1: Impact du climat et de l'habitat sur la phénologie des populations de lézard vivipare

Dans ce premier chapitre, j'ai voulu estimer l'impact des variations climatiques locales sur la date de mise bas. La date de mise bas est un trait phénologique fréquemment analysé car facile à mesurer, sous sélection et connu pour être plastique chez de nombreuses espèces (Brommer *et al.*, 2008a). Chez le lézard vivipare, plusieurs études ont déjà démontré que la date de parturition est un trait influencé par les conditions thermiques de l'environnement de gestation (voir Introduction). Ici, je me suis plus particulièrement intéressé à caractériser les facteurs pouvant influencer l'intensité de cette relation plastique en comparant les normes de réactions de différentes populations du suivi 'Global Change'. De telles études empiriques existent, mais celles réalisées également en populations naturelles sont encore rares (Husby *et al.*, 2010b; Porlier *et al.*, 2012). Pourtant des études réalisées en populations naturelles démontrant un lien entre climat et intensité de la plasticité phénotypique d'un trait sont primordiales pour mieux comprendre l'effet de l'environnement sur l'évolution de la plasticité. De nombreux modèles démontrent par exemple que la forme des normes de réactions est liée à la prédictibilité et à l'hétérogénéité des conditions environnementales (Chevin *et al.*, 2012).

Dans ce travail, j'ai caractérisé 11 des populations du suivi par leur température moyenne, la variance des températures au cours de la gestation et par leur degré d'anthropisation. Mes résultats démontrent des effets combinés de chacun de ces facteurs sur la date de parturition, avec un avancement des dates de pontes les années chaudes, particulièrement prononcé dans les populations anthropisées. De plus, les résultats illustrent l'influence de l'hétérogénéité environnementale sur l'intensité de la norme de réaction et confirment en partie les attendues théoriques en populations naturelle.

Article 1: Climate and habitat interact to shape the thermal reaction norms of breeding phenology across lizard populations

Journal: Journal of Animal Ecology, *in press*

Authors : Alexis Rutschmann, Donald B. Miles, Jean-François Le Galliard, Murielle Richard, Sylvain Moulherat, Barry Sinervo & Jean Clobert

Abstract :

1. Substantial plastic variation in phenology in response to environmental heterogeneity through time in the same population has been uncovered in many species. However, our understanding of differences in reaction norms of phenology among populations from a given species remains limited.
2. As the plasticity of phenological traits is often influenced by local thermal conditions, we expect local temperature to generate variation in the reaction norms between populations.
3. Here, we explored temporal variation in parturition date across 11 populations of the common lizard (*Zootoca vivipara*) from four mountain chains as a function of air temperatures during mid-gestation. We characterized among-population variation to assess how local weather conditions (mean and variance of ambient temperatures during mid-gestation) and habitat openness (an index of anthropogenic disturbance) influence the thermal reaction norms of the parturition date.
4. Our results provide evidence of interactive effects of anthropogenic disturbance and thermal conditions, with earlier parturition dates in warmer years on average especially in open habitats.
5. Variation in the reaction norms for parturition date was correlated with mean local thermal conditions at a broad geographical scale. However, populations exposed to variable thermal conditions had flatter thermal reaction norms.
6. Assessing whether environmental heterogeneity drives differentiation among reaction norms is crucial to estimate the capacity of different populations to contend with projected climatic and anthropogenic challenges.

Key-words: among-population variation, anthropogenic disturbance, common lizard, parturition date, phenology, plasticity, reaction norm, thermal sensitivity

La version finale du manuscrit est disponible en Annexe 4

INTRODUCTION

Changes in phenology, the timing of life cycle events, due to climate warming have been well documented during the past decades. A shift in phenology, in particular the timing of breeding event, is often considered to be an effective response by which plants and animals can cope with climate warming (Walther *et al.*, 2002). Substantial evidence suggests that phenological traits exhibit plasticity within populations and fluctuate from year to year to track prevailing environmental conditions (Menzel & Fabian, 1999; Réale *et al.*, 2003; Visser & Both, 2005; Charmantier *et al.*, 2008). For example, a recent meta-analysis estimated that the current increase in temperatures has advanced the phenology of reproduction and migration in 62 % of 678 species (Parmesan & Yohe 2003). The extent to which a species phenology may respond to climate warming throughout its range depends however on spatial variation in the sensitivity of phenology to local climate conditions (Chevin *et al.* 2010, Chown *et al.* 2010). Yet, most shifts in phenology have been observed only within a single natural population. Our current knowledge of temporal variation in phenology across spatially distinct populations is limited to a few long-term studies of breeding dates in avian populations (Visser *et al.*, 2003; Both *et al.*, 2004; Brommer *et al.*, 2005; Nussey *et al.*, 2005; Husby *et al.*, 2010; Porlier *et al.*, 2012; Visser *et al.*, 2012) and one study on mammals (Sheriff *et al.* 2011)

An efficient way to explore plasticity in breeding phenology is the study of the "reaction norm" that expresses variation of the breeding dates along an environmental gradient, such as thermal conditions (Brommer *et al.*, 2005; Nussey *et al.*, 2005; Garant *et al.*, 2008). Comparison of the thermal reaction norms for breeding date among populations estimates the extent of spatial variation in phenotypic plasticity. Studies of multiple populations will also provide insights into the causes of variation in thermal reaction norms. Moreover, the understanding of variation in phenology among populations is a unique opportunity to predict how populations should respond to climate change given the anticipated exposure of species to novel combinations of climatic and anthropogenic conditions (Travis, 2003; Baumann & Conover, 2010; Chown *et al.*, 2010).

Several studies have highlighted the potential for a strong selection on the timing of breeding phenology (Chaine, 2010). First, energetically demanding activities associated with breeding should coincide with periods of suitable food availability, and match the temporal dynamics of prey species in the environment (match-mismatch hypothesis, Sinclair & Tremblay, 1984; Miller-Rushing *et al.*, 2010; Visser *et al.*, 2012). Second, phenological traits are often heritable and exhibit significant levels of additive genetic variation, suggesting

potential for an evolutionary response to selection and the local adaptation of breeding phenology traits (Scheiner, 2002; Nussey *et al.*, 2005; Bradshaw *et al.*, 2006). Different evolutionary and ecological scenarios make different predictions about the degree of variation in breeding phenology reaction norms across populations (Fig. 1). First, breeding phenology may vary across populations even if they share the same reaction norm because populations have different positions on the environmental gradient (Fig 1a). Second, breeding phenology may not differ across populations despite environmental variation because selection promotes physiological or behavioral adaptations such that individuals breed on average at the same dates (Fig 1b). Finally, populations may evolve a locally adapted reaction norm, influenced by prevailing local conditions, and thus display a locally adapted response (Fig 1c).

When breeding phenology shows a linear relationship with environmental conditions, differences in phenotypic plasticity across populations may be assessed by analyzing the magnitude of the intercept and slope of the reaction norm (Baumann & Conover, 2010; Baythavong & Stanton, 2010; Husby *et al.*, 2010). The extent and the causes of variation in the slope of reaction norms for breeding date remain uncertain, but some evolutionary theory predicts that phenotypic plasticity should be greater, and therefore the reaction norm should be steeper, in populations occupying sites characterized by large, but predictable, temporal, environmental variation (Ghalambor *et al.*, 2007a; Chevin *et al.*, 2010). A comparative analysis of multiple populations inhabiting heterogeneous environments should thus provide a unique opportunity to identify putative causal factors of selection on phenology plasticity (Husby *et al.*, 2010b; Porlier *et al.*, 2012; Klepsatel *et al.*, 2013).

Squamate reptiles are ideal models to characterize reaction norms of breeding phenology because of their dependence of key physiological processes to body temperature (Huey & Stevenson, 1979; Clusella-Trullas *et al.*, 2011; Le Galliard *et al.*, 2012). Although the effect of climatic conditions on body temperatures may be buffered by compensatory responses of basking behavior, most physiological performance traits of ectotherms are sensitive to climate conditions, which should affect importantly their fitness (Huey *et al.*, 2003, 2009; Kearney *et al.*, 2009; Sinervo *et al.*, 2010; Clusella-Trullas *et al.*, 2011). Here, we analyzed variation in parturition date within and among populations of the common lizard (*Zootoca vivipara*). This lizard is an obligate hibernating species and all reproductive stages (vitellogenesis, ovulation/fertilization and gestation, and energetic recovery) take place between early May to late September. Previous studies have demonstrated earlier parturition dates in warmer years (Chamaille-Jammes *et al.*, 2006; Lepetz *et al.*, 2008; Le Galliard *et al.*,

2010), but the inter-population variation in reaction norms with respect to habitat variation has not yet been characterized.

In this study, we determined how environmental conditions affects parturition date using a sample of 11 populations from the Massif Central, France ranging from 1200 to 1450 m a.s.l. This set of population includes variation in both climatic and anthropogenic conditions encountered by common lizards in this area. The region represents the southern margin of the distribution of the common lizard and local climatic conditions are heterogeneous depending on elevation and prevailing winds. Moreover, the region has a long history of pastoralism and our set of populations present different levels of human mediated alterations. To estimate the impact of such heterogeneity on the reaction norm of parturition phenology, we addressed the following questions: 1) is local variation in parturition date affected only by climatic factors?; 2) are the local reaction norms affected differently by the thermal gradient experienced by the populations?; 3) how is the variation in the reaction norms of parturition date structured according to prevailing weather and habitat conditions?

MATERIAL AND METHODS

Natural history of *Zootoca vivipara*

The common Lizard *Z. vivipara* is a widely distributed lizard species in the family Lacertidae from Eurasia. Most of the European and Asian populations are ovoviviparous except for egg-laying populations inhabiting the very southern margin of the distribution (Surget-Groba *et al.*, 2001). In our study area, adult males emerge on average from hibernation in April, before yearlings and adult females. Females emerge in late April- early May. Mating happens immediately after females' emergence. Egg fertilization is initiated shortly after copulation and gestation occurs between the beginning of May and the middle of July. The onset of parturition may be as early as in mid-late July and last through early August. After parturition, females accumulate energy reserves until hibernation which starts on average in October (detailed life cycle and phenology in Bleu *et al.* (2013)).

Population monitoring and breeding conditions

The focal populations are located in four mountain chains from the Massif Central, France (exact locations and map in Table 1 and Fig. 2). We sampled 11 populations during June every two years (i.e. on average 5 capture episodes per population) from 2003 to 2014.

However, some populations were sampled as few as 3 different years and others up to 6 different years (Table 1). Average date of capture was the 27th of June (s.d. = 3.5 days). The variation in date of capture was mainly due to poor weather conditions that delayed the efficiency of captures. At each sample interval, we captured 15 to 20 pregnant females from each population, and measured their snout to vent length (SVL, mean = 60.1 ± 4.9 mm) and body mass (mass = 4.76 ± 1.08 g). Pregnant females were brought to the laboratory and individually housed in an individual terrarium under standardized conditions (11x18x11 cm, average temperature = 25.19°C ; s.d. = 2.31) until parturition (Massot & Clobert, 2000a). The temperature on the field while females were kept in captivity is was on average 22.2°C (s.d.= 0.83) but varied between 16.56°C (s.d. = 1.23) in the colder population and 24.81°C (s.d. = 3.21) in the warmer one. Females were exposed to the local prevailing photoperiod, but provided with access to heat for 6 hours per day (two times 3 hours, once in the morning and one in the afternoon). Female mortality during captivity varied between 1 and 3% among years. Parturition date (PD) was calculated from the first of January (e.g. 1 = January 1st; mean PD = 199; s.d. = 7 days; or 18 July $\pm$ one week). Three days after parturition, each female was released with her offspring at the exact capture location. Across the 11 years of study, the females spent on average 22.4 days (s.d. = 6.9 days) in captivity, which represents approximately between 25 and 30% of a gestation period of approximately two and a half months.

Description of local weather data and habitat quality

Air temperatures for each population were obtained from the national French meteorological agency data-base (MeteoFrance, MF, <http://publitheque.meteo.fr>). To explore the impact of temperature on parturition date, we used the mean maximal temperature of the month of June (T_{max6}). June temperature has been shown as the best proxy of thermal conditions during gestation and is known to influence significantly the length of gestation in several populations (Chamaille-Jammes *et al.*, 2006; Lepetz *et al.*, 2009; Le Galliard *et al.*, 2010). Moreover, Lourdais *et al.* (2004) showed that the period of development the most sensitive to temperature is the middle part of gestation in the oviparous snake (*Vipera aspis*). For the common lizard, this middle stage of gestation correspond to June (Bleu *et al.*, 2013). To match this period as close as possible when calculating the T_{max6} that characterize each study site, we only used the days females had experienced the ambient temperatures (that is a substantial fraction of the month of June) and removed the days they spent in the laboratory.

We calculated local temperatures for each population from the closest MF meteorological station. However, we were unable to find a unique MF station for all populations (Table 1). As a consequence, to fit better the local conditions from MF data, we corrected MF's recordings based on an empirical calibration curve. Temperature data loggers (Thermochron iButtons[®]) were placed on the ground at each capture localities from mid June 2014 to the end of July 2014. The temperatures recorded by logger for each population were linearly related to those recorded by the best, nearby meteorological stations (see Figure S1). We used the regression coefficients from the linear regression analysis to predict climatic conditions for each population from MF data (see details in Table S1). Despite the significant altitudinal gradient present in our study, we did not use elevation in our statistical models because T_{max6} was a more relevant descriptor of breeding phenology variation than altitude (results not shown). We are not able to exclude the possibility that other proxies than T_{max6} are better in some populations but the fit of our reaction norms is satisfactory in most cases. Our approach for calibrating the MF data allows us to use a common model to analyze variation among the eleven studied populations. Finally, we also calculated an index of Forest Cover (FCI) to assess the effects of anthropogenic activities and thus local habitat quality. In the study populations, the primary source of disturbance is grazing by livestock. Using aerial photographs (scaled Google Earth[®] views), we measured FCI as the proportion of the number of pixels representing trees or bushes in the total capture area.

Among population variation in reaction norms

To study reaction norms of parturition dates (PD) across populations, we performed a three-step analysis. First, we tested whether geographical proximity between populations was important by nesting each population within their individual mountain chain in a random regression model with population as a random factor. The goodness of fit of this model was then compared using the corrected Akaike Information Criteria (AICc) to the one of a model excluding the effect of local mountain chain and the one of the null model. Second, using a linear random regression models, we tested patterns of variation in the reaction norms among populations. The full model included fixed effects of T_{max6} , T_{max6}^2 (to account for a potential non-linearity), FCI, SVL (like in previous studies) and first-order interactions between temperature variables and FCI. All variables were centered and scaled by their standard deviation to facilitate the interpretation of model estimates. We searched for both inter-population differences in average value of parturition date (intercept of the reaction norms)

and in the response to variation in T_{max6} (steepness of the slope) by including random effects of population on the intercept only or on both slope and intercept of the model:

<i>Minimal model :</i>	$PD \sim \text{fixed effects}$
<i>Random intercept :</i>	$PD \sim \text{fixed effects, random} = \text{Population}$
<i>Random intercept and slope</i>	$PD \sim \text{fixed effects, random} = \text{Population} + T_{max6} * \text{Population}$

We first evaluated the random part of the model using AICc selection and a Likelihood Ratio Test (LRT). Then, we assessed the significance of fixed effects using backward selection of non-significant terms (threshold of significance < 0.05 , Zuur *et al.*, 2009).

Last, given significant variation in the thermal reaction norms (see below), we assessed whether the position of each population along the thermal and habitat gradient influenced the intercept and slope coefficients. We retained the regression coefficients of a linear model linking the parturition date PD to T_{max6} for each population after temperatures were centered on the mean temperature of the eleven populations. Coefficients of the reaction norms were then regressed against the local mean T_{max6} (mean value from each population), FCI, and variance in T_{max6} (VT_{max6}) using multiple linear regression models. We used two variables to represent two different aspects of climatic conditions: T_{max6} represent mean conditions of mid-gestation for each population, while VT_{max6} scores the temporal heterogeneity of weather conditions during the 11 years of sampling. However, their values were positively correlated (Spearman correlation coefficient, $r = 0.574$). To check for some co-linearity issues, we calculated the Pearson's bivariate correlations predictor ($|r| = 0.67$) and the variance inflation factor ($VIF = 2.00$). These values were both under the traditional thresholds ($|r| < 0.7$ and $VIF < 5$, respectively) suggesting that co-linearity between the 2 variables could be neglected in a multiple regression. As a consequence, we included both variables to determine the impact of climatic conditions on the shape of the reaction norms and employed backward elimination of the non-significant variables to select for the best model.

RESULTS

Average T_{max6} (the mean maximal temperature of June) during the sample period 2003-2014 varied from 15.85°C to 22.86°C. Our index of disturbance, FCI, varied from 0% forest cover (fully grazed habitat) to 32% (mesic, mixed open habitat, see Table1). Average parturition date (PD) varied between day 195 and 203 across populations. We found no evidence of a

trend towards warmer conditions during the time period of this study (10 years); instead, all populations showed a weak decrease of temperature between 2003 and 2014 (Fig S1). Females captured from populations located in the "Mont d'Aubrac" mountain chain had earlier parturition dates despite cooler ambient temperatures. However, according to ΔAIC differences, the model with a random population effect ($AICc = 6638.8$) outperformed the nested model with a mountain chain effect ($AICc = 6782.3$) and the null model ($AICc = 6713.9$) meaning that populations differences override the differences among mountain chains. Thus, we used the model with a random population as a starting point in our analysis of the reaction norms.

The random regression models showed significant inter-population differences in both the mean and slope of the reaction norm ($AICc$ minimal model = 6472.5, $AICc$ random intercept model = 6134.1, $AICc$ random intercept and random slope model = 5986.1). Selection of fixed effects led to a final model summarized in Table 2. First, females with a larger SVL had earlier parturition dates (slope $b = -0.91 \pm 0.15$; $t_{1032} = -5.98$; $P < 0.0001$). In addition, PD was significantly advanced by T_{max6} ($b = -5.88 \pm 2.54$; $t_{1032} = -2.31$, $P = 0.021$) and was influenced by a two-way interaction between T_{max6}^2 and FCI (Table 2). The exact pattern was that the PD is shortened by T_{max6}^2 when FCI equals zero ($b = -2.92 \pm 0.41$; $t_{1032} = -7.12$; $P < 0.0001$), PD is delayed by FCI when T_{max6}^2 equals zero, i.e. climate conditions are average ($b = 4.94 \pm 2.19$; $t_9 = 2.25$; $P = 0.050$), and the effect of FCI on the delay of PD is reduced at increasing values of air temperatures in June ($b = -1.41 \pm 0.62$; $t_{1032} = -2.19$; $P < 0.023$).

We estimated local reaction norms by performing a linear regression between PD and local temperature for each population (see Table S1 for details of regression estimates). All reaction norms exhibited a negative relationship between mid-gestation temperatures and PD (Fig. 3). The one exception was BOB, which had a positive association. We found a significant and positive effect of VT_{max6} on the slope of the reaction norms ($b = 2.281 \pm 0.83$; $t_8 = 2.74$, $P = 0.0254$; Fig. 4b). We also found a significant and positive effect of VT_{max6} on the elevation of the reaction norm ($b = 5.89 \pm 0.95$; $t_8 = 6.190$; $P = 0.0002$; Fig. 4e). Finally, we note that the USA population was characterized by particularly cold climate conditions and its reaction norm was a significant outlier that might influence importantly the relationship between coefficients of the reaction norm and environmental conditions. However, the qualitative and quantitative results remain unchanged when we excluded USA of the dataset (see details in the caption of Fig. 4).

DISCUSSION

In the present study we confirmed, at a large geographic scale, that populations of the common lizard advanced their parturition date (PD) in years with warmer temperatures during the mid-gestation period (June). We also found that PD is influenced by the interaction between variation in climatic and anthropogenic disturbance as assessed by the extent of grazing. Finally we demonstrated that PD reaction norms vary among several common lizard populations and that differences in both the mean and sensitivity of PD to thermal conditions are correlated with local climatic conditions.

Impact of local conditions on phenology

Parturition dates of *Z. vivipara* in the Massif Central were generally earlier when females experienced warmer temperatures during mid-gestation inside their natural populations. This pattern was evident across all populations and is consistent with previous findings, which demonstrated that females from a single population on Mont Lozère have specific thermal requirements for gestation during June that are more difficult to reach in cooler years (Le Galliard *et al.*, 2010 and Chamaillé-James *et al.*, 2006). In our study, we extend this result by showing that local, climate effects rather than global, climate trends are crucial when modeling phenological reaction norms across populations at a regional scale. Indeed, some populations included in our sample were clustered in mountain regions and sometimes only few kilometers apart (Fig. 2). However, local conditions, and in particular thermal conditions, were stronger determinants of PD than spatial proximity. In addition, these effects of climate conditions were seen despite the fact that females were brought to the laboratory in warm and homogeneous conditions. Laboratory conditions may bias estimates of PD reaction norms by buffering differences among individuals and populations since delayed individuals and populations may catch up under *ad libitum* conditions. Thus, despite of the caution to sample the females late in the gestation (when thermal effects are less critical, Lourdais *et al.* 2004), we may have underestimated the magnitude of breeding phenology plasticity.

Our results also show that anthropogenic activities may not only have direct effects on the breeding phenology, but interact with the effect of ambient temperature as suggested by the significant interaction between temperature effects and the forest cover index. This interaction exacerbates the effects of ambient temperatures during mid-gestation and may be a consequence of the differences in the ability of common lizards to cope with temperature in fully open versus partially covered habitats. Our analyses suggest there are stronger effects of

temperatures in open, i.e., heavily grazed, areas. Economic activity in the Massif Central is, in large part, dependent on meat and milk production, pastoralism and agriculture. In the next few decades, the increase in temperatures and the new economic dynamic in the region by promoting livestock production hence grazing activities, should lead to an increasingly disturbed and open landscape, enhancing the effects revealed in our analyses. Moreover, different species of livestock (e.g., cows, sheep, horses) do not pose a direct threat to lizards, but may have severe indirect effects by disturbing basking behavior, reducing the availability of thermal refuges or by carrying parasites that may affect lizard performance.

It is difficult to assess the fitness consequences of accelerating embryogenesis because shifts in parturition date can have both positive or negative consequences on fitness components in common lizards. On the one hand, earlier PD should enhance the growth rate of neonates (Bestion *et al.*, in prep.) and increase offspring survival (Le Galliard *et al.*, 2010), as well as maternal survival (Chamaille-Jammes *et al.*, 2006). Yet, a shorter gestation period is also linked with warmer temperatures that, if associated with drought, could be lethal for neonates, as experimentally demonstrated by Bestion *et al.* (in prep). All things considered, we can expect a mixture of habitat and climatic factors to not only impact phenology as shown in our analysis but to affect population dynamics and age structure (Townroe & Callaghan, 2014), individual parasite loads (Pounds *et al.*, 2006) and species distribution (Krockenberger *et al.*, 2012).

The negative quadratic effect of temperature during gestation on mean parturition date across populations suggests an accelerating effect of thermal conditions on the speed of gestation. High body temperature is known to enhance physiological performance in ectotherms up to a given temperature threshold, called optimal temperature (T_o) for performance (Huey & Stevenson, 1979). This non-linear sensitivity to temperature could be an hypothesis to explain the pattern of accelerating progressively gestation timing. Because sampling opportunities in our study system was limited to a maximum of 6 parturition years, it is challenging to disentangle whether the observed non-linearity was due to non-linear intra-population plasticity rather than differences in reaction norm slopes among populations (see Fig. 3). Detailed data from a long-term study of one population of *Z. vivipara* on Mont Lozère monitored by capture-recapture protocols continuously from 1986 to 2012 (see Massot *et al.*, 2008 for a complete description) offered a unique opportunity to test for the existence of a quadratic, decelerating reaction norm in the same population. Supplementary analyses based on this population showed that the temperature during gestation ranged from 18.4 to 25.1°C, but that parturition date was linearly, and not quadratically, related to mid-gestation

temperature in this population (see Fig. S3 and Table S4). This result suggests that the non-linearity observed in our large sample of populations may be a consequence of differences among populations rather than a truly, non linear plasticity (see next section).

Among population variation in reaction norms

One goal of our study was to explore the environmental characteristics that shape among-population variation in the reaction norm for parturition date including mean thermal conditions, variance in thermal conditions and forest cover. We found that consistent exposure to warm temperatures advanced differently parturition dates according to local weather conditions. In contrast with theoretical predictions (Ghalambor *et al.*, 2007; Chevin *et al.*, 2010), parturition dates decreased more quickly with local temperature in populations characterized by a lower variance in mid-gestation thermal conditions. These results are surprising as they show a significant negative relationship between the magnitude of the plasticity and environmental heterogeneity. In other words, sites with low inter-annual variation in mid-gestation conditions show the strongest response to climate (lowest negative slope of the reaction norm), whereas sites with higher variation display weaker responses (flatter reaction norms; see Fig. 3 and Fig. 4). This result is of particular importance in the context of current global change which may enhance variation in weather conditions and alter environmental stability (reference). Indirect effects of climate change may include divergent selection on breeding phenology plasticity and could therefore impact the parturition dynamic of the common lizard.

The observed divergence in the magnitude of the plastic response may partially be explained by the predictability of ambient conditions during mid-gestation rather than the variance *per se*. The few studies that have compared PD reaction norms between different populations of a same species (Lind & Johansson, 2007; Husby *et al.*, 2010a; Porlier *et al.*, 2012) and some theoretical models (Ghalambor *et al.*, 2007; Chevin *et al.*, 2010) suggest that unpredictable environments will impede the evolution of plasticity as the probability to produce a maladapted (or miss-matched) response is enhanced when heterogeneity is too great. In our case, environments with higher levels of variability in mid-gestation temperatures may be less predictable; however, it is difficult to statistically assess the predictability of the climate with available statistical methods and we cannot confirm that predictability is the factor that drives the relation. A second hypothesis might be that females are more responsive in colder populations. For example, populations with the least variable

thermal conditions during gestation tend to be the coldest in our data set (Fig. 3e, marginal correlation: $P=0.11$). The observed differences could thus reflect local adaptation that enhances the ability of females to benefit from advantageous thermal conditions when they arise. Indeed, a shortened gestation period may have been selected to release the pressure on the post-gestation period that drives preparation to hibernation of the adults and the juveniles (Bestion *et al.*, *in prep*).

In conclusion, our results (a) provide evidence of interactive effects of anthropogenic disturbance and climatic conditions on parturition date, (b) suggest that variation in PD reaction norms is correlated with local environmental conditions at a broad geographical scale and (c) indicates that local climate variation is a major driver of spatial variation in breeding phenology plasticity. Altogether, these results emphasize the need of considering multiple factors that could drive local adaptation of reaction norms (i.e. temperature, human activities) when trying to assess how local adaptation may help populations to cope with climate change. Our study also underlines the need to take into account variation in reaction norms in the management strategy of populations threatened by climate warming and caution against the use of stable thermal reaction norms to predict the future ranges of species with climate niche models.

ACKNOWLEDGMENTS

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Figure 1. Evolutionary scenarios to explain potential differences among populations in their plastic response. Each point represent the observed response of a population. Solid lines represent among population reaction norms, dashed lines represent population reaction norm. (1a) Populations share a common reaction norm but present local differences in their phenotypes as they are spread at different position on the environmental gradient. Among populations and populations reaction norms are confounded. (1b) Populations share a common reaction norm but do not present any differences as selection promotes physiological or behavioral adaptations such that individuals breed on average at the same dates. (1c) Each population evolve a locally adapted reaction norm influenced by local conditions.

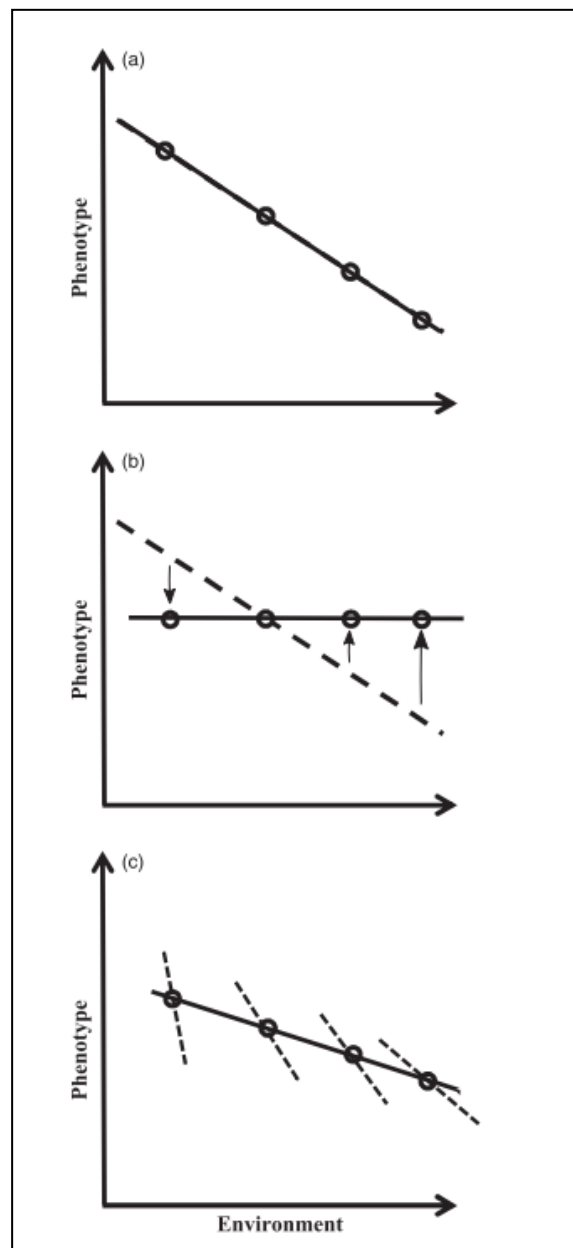


Figure 2. Location of the common lizard populations in the Massif Central, France. The white line circumscribes the Massif Central. The dashed lines represent the southern margin of viviparous common lizard distribution. Localities of the study populations: BES = Lou Bes, BEL= Bel Air, BOB = Bout de la Barre, BON= Col de Bonnecombe, BOU = Baraque du Bouvier, COM= Col du Cheval Mort, COP= Col du Pendu, JON = Gerbier des Joncs, PAR = Lou Paradis, TIO = Tioule, and USA= Usanges.

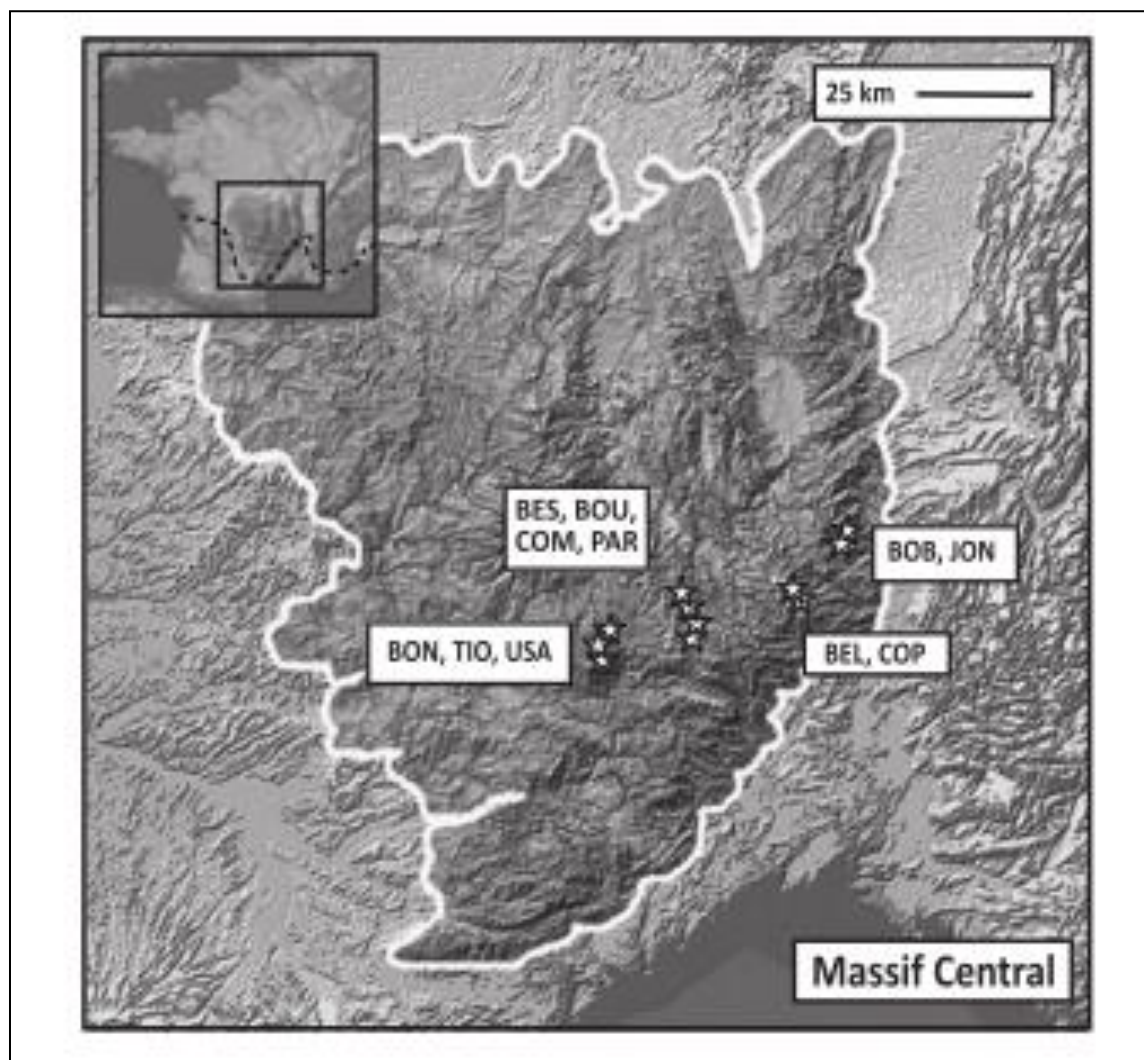


Figure 3. Population-level reaction norms in each study site of global survey, described as the relation between parturition date and average temperature in mid-gestation (T_{max6}). All relationships were significant ($p < 0.05$; with the exception of BES $p = 0.06$). We excluded one population from the analysis (BOB) as the relation was positive, which may reflect insufficient sampling opportunities.

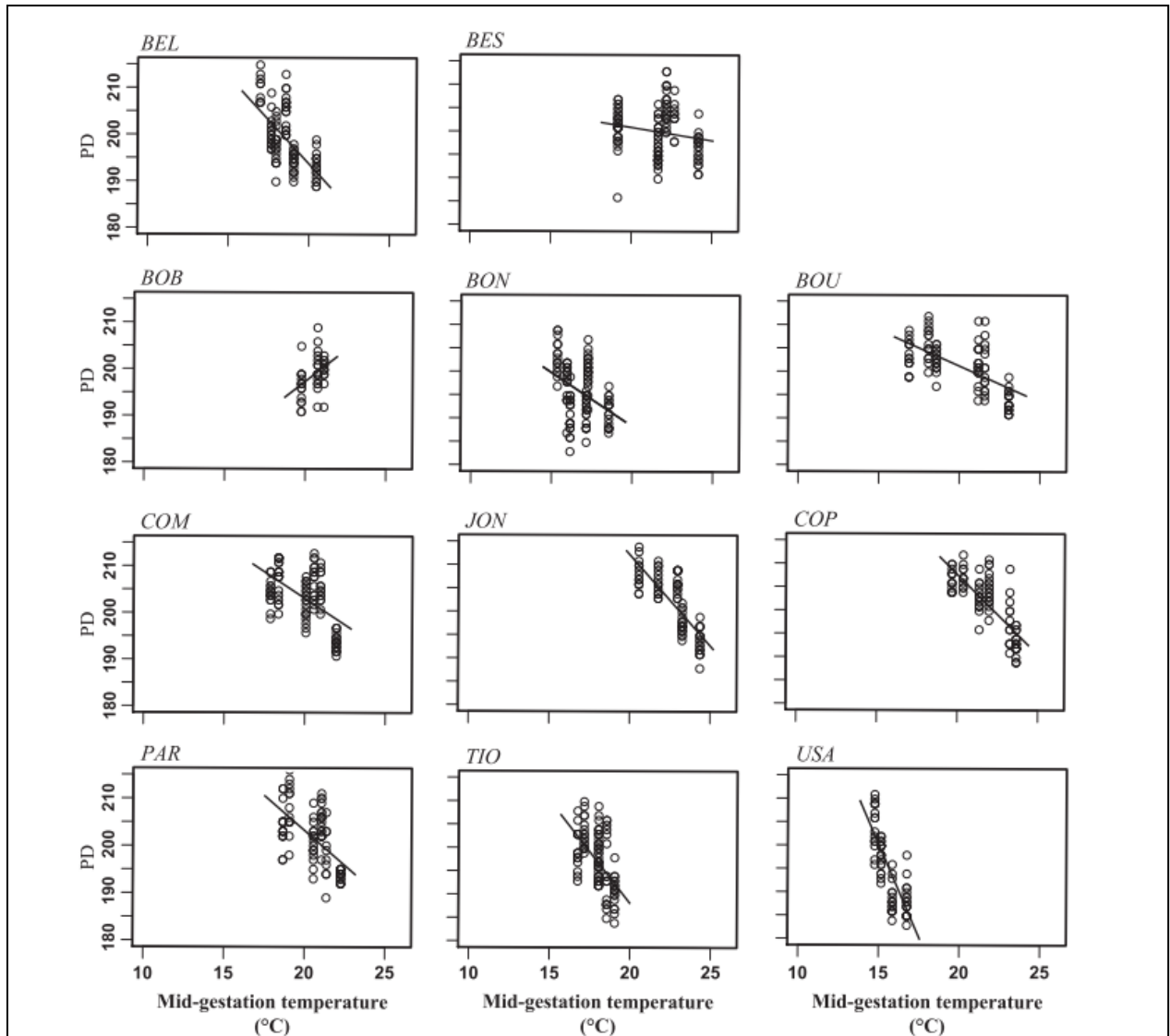


Figure 4. Slope of the populations reaction norms ("Regression slope"; a., c., e.) and intercept ("Regression intercept"; b., d., f.) against temperature during mid-gestation (a. & b.), Forest Cover Index (c. & d.) and Variance in Mid-gestation temperatures (e. & f.). Solid lines represent significant relationships ($P < 0.05$), dashed lines represent non-significant relationships. We note that the USA population may drive observed relationships. However, the population experiences cooler environmental conditions than other population, because of the constant windy. We hypothesize that this extreme environment is relevant to consider when comparing phenotypes among different. However, we repeated our analyses with USA removed from the dataset and the results remained unchanged (The slope of the reaction norms remain correlated with $VT_{\max 6}$, $P = 0,034$; and the elevation is still influenced by $T^2_{\max 6}$, $P = 0,004$).

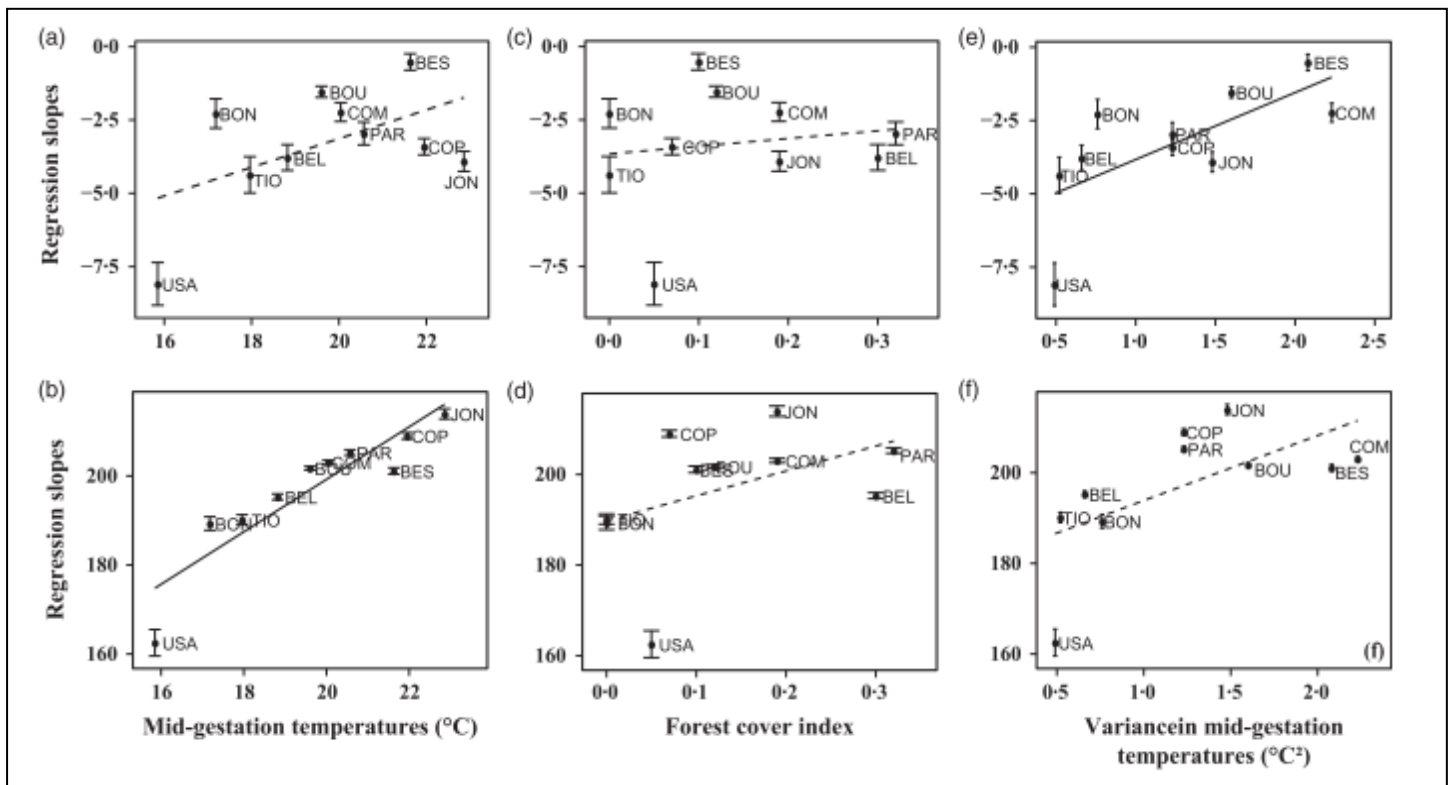


Table 1. Position and description of each population with associated mountain chain, sampling opportunities, elevation, forest cover index (FCI), meteorological station and estimated mean daily maximal temperature in June (T_{max6}).

Name	Longitude	Latitude	Mountain Range	Number of samples	Meteorological station	Elevation	FCI	T_{max6}
BEL	44°40'3.32N	4°01'5.50E	Mont du Velay	7	Loubaresse	1385m	0.30	18.82
COP	44°39'3.02N	4°01'7.82E	Mont du Velay	6	Loubaresse	1392m	0.07	21.95
BES	44°35'2.81N	3°30'4.81E	Margeride	7	Mende	1200m	0.1	21.63
COM	44°40'0.14N	3°31'9.14E	Margeride	6	Mende	1401m	0.19	20.04
PAR	44°36'3.06N	2°29'4.79E	Margeride	6	Mende	1454m	0.32	20.57
BOU	44°45'4.28N	2°28'9.05E	Margeride	5	Mende	1250m	0.12	19.60
TIO	44°35'2.43N	3°06'3.37E	Mont d'Aubrac	6	Nasbinals	1272m	0	17.96
USA	44°38'5.45N	3°07'2.33E	Mont d'Aubrac	4	Nasbinals	1219m	0.05	15.85
BON	44°33'5.63N	3°07'6.47E	Mont d'Aubrac	6	Nasbinals	1344m	0	17.18
JON	44°50'0.98N	4°12'6.49E	Mont du Vivarais	5	Mazzan Abbaye	1381m	0.19	22.86
BOB	44°49'4.50N	4°13'6.96E	Mont du Vivarais	3	Mazzan Abbaye	1430m	0.04	21.02

Table 2. Best linear mixed effects model summary table for parturition date versus snout to vent length (SVL), forest cover index, mid- and squared mid-gestation conditions (T_{max6} , T^2_{max6}) and first interaction terms. Temperature was nested within populations as a random effect.

Predictor Variables	Estimate	Std. Error	d.f.	t-value	P-Value
Intercept	196.96	3.88	1032	50.74	<0.0001
SVL	-0.91	0.15	1032	-5.99	<0.0001
FCI	4.94	2.19	9	2.26	0.050
T_{max6}	-5.89	2.55	1032	-2.31	0.006
T^2_{max6}	-2.93	0.41	1032	-7.13	0
CFI * T^2_{max6}	-1.41	0.62	1032	-2.27	0.042
Random effects	Std. Dev.	Corr			
Population (intercept)	12.79				
Population (slope)	4.52	0.84			

CHAPITRE 2 : Influence du climat sur le Trade-off entre la taille de portée et la qualité des juvéniles.

Après avoir démontré à quel point la plasticité phénologique des individus était liée aux conditions du milieu dans lequel ils évoluent, j'ai voulu explorer un aspect des traits d'histoire de vie plus directement lié à la valeur sélective des individus. J'ai choisi d'étudier la relation antagoniste (ou trade-off) qui existe entre la qualité de la portée d'une femelle et le nombre de juvéniles produits. La quantité d'énergie qu'un individu peut investir dans sa reproduction est limitée et un choix (un compromis) s'impose logiquement entre le nombre de petits que l'on peut produire et leur qualité. Ce trade-off est un classique de la biologie évolutive et il a déjà été étudié sous de nombreux angles. Cependant, en fonction de l'environnement, les patrons de sélection agissant sur chaque trait impliqué dans la relation peuvent varier, accentuant ou non les contraintes à l'origine de ce compromis (Roff & Fairbairn, 2007).

En utilisant les données du projet 'Global Change', j'ai pu estimer l'intensité du trade-off chaque année de suivi, pour chaque population. J'ai ensuite mis cette intensité en relation avec les conditions thermiques rencontrées localement par les femelles. Pour la majeure partie des populations, les résultats indiquent que le trade-off s'atténue avec l'augmentation des températures, signe d'une disparition des contraintes qui en sont la cause. Cet effet positif de la température se retrouve aussi à une échelle inter-population puisque les populations les plus froides montrent moins de flexibilité dans le trade-off, qui reste stable malgré des variations de températures. Mis en perspective avec d'autres travaux effectués sur cette espèce, ces résultats suggèrent une potentielle accélération du cycle de vie du lézard vivipare lorsque les températures du milieu augmentent menant à un potentiel déclin des populations.

Article 2: Warmer temperatures suppress the classic offspring quality and number trade-off in the common lizard, Zootoca vivipara.

For consideration in: Biology Letters*, *under consideration*

Authors: Alexis Rutschmann, Donald B. Miles, Jean Clobert & Murielle Richard

Abstract:

Life history traits involved in trade-offs are known to vary with environmental conditions. Here, we have evaluated the response of the "offspring number vs quality" trade-off to ambient temperature in 11 natural populations of the common lizard. We provide evidence at both the intra and inter-population level that the trade-off is reduced, and in some instances eliminated, with an increase in air temperature. If this effect enhances current individual fitness, it may lead to an accelerated pace of life in warmer environments and could ultimately increase adult mortality. In the actual context of global warming, our results advocate more studies in natural populations to explore trade-offs between life-history traits.

Key-words: Trade-off; Offspring quality; Clutch size; Thermal gradient; Life-history traits; *Zootoca vivipara*

**Biology Letters operates a strict word limit of 2500 words for Research articles*

INTRODUCTION

Trade-offs among reproductive traits represent a fundamental principle of life history theory (Stearns, 1992). Since females have a finite amount of energy available for reproduction, they have to make crucial decisions regarding the optimal allocation strategy to adopt. For over 50 years, numerous studies have attempted to explain observed trade-offs between survival and reproduction, current and future reproduction, or number and quality of offspring (e.g. Kirkwood and Rose 1991). In most situations, negative correlations emerge as a female cannot simultaneously optimize all life history traits. However, null or positive correlations are also found, suggesting that constraints on life history traits can potentially be released (Service & Rose, 1985; Roff & Fairbairn, 2007).

Patterns of selection acting on life history traits are known to vary between habitats (Czesak & Fox, 2003; Sgrò & Hoffmann, 2004). For example the balance between number and size of offspring may depend on the ecological context: females should produce better offspring if juvenile survivorship is poor but may enhance their litter size in high quality environments (Fox *et al.*, 1997). As a consequence, interactions between traits may circumstantially be magnify, diminish or obviate along environmental gradients. The influence of environmental conditions on the intensity of trade-offs has been considered in theoretical works (Van Noordwijk & de Jong, 1986; Roff & Fairbairn, 2007) or tested experimentally (Czesak & Fox, 2003) but studies of natural populations are still scarce (Donohue *et al.*, 2000).

In the present work, we examined how trade-offs between litter size and juveniles quality varied across 11 populations of the common lizard *Zootoca vivipara* in the Massif Central (France). Maternal effects are known to vary among lizard populations according to environmental conditions (Swain & Jones, 2000), and particularly for the common lizard (Meylan & Clobert, 2004; Bleu *et al.*, 2013). Here, the sampled populations, distributed along a thermal gradient, allowed us to address the following questions regarding the intensity of the trade-off: 1) Is there any evidence of variation between populations? 2) If so, is this variation structured according to thermal characteristics?

METHODS

The common lizard (*Zootoca vivipara*, Lacertidae) is widely distributed across Eurasia. Mating takes place in May, immediately after females' emergence and parturition occurs between mid-July and early August. Between August and October females and juveniles accumulate energy to prepare hibernation (Bleu et al 2013).

We obtained lizards from 11 populations in the Massif Central (France), between 2003 and 2015 (Rutschmann et al. 2015). We captured between 15 to 30 females at each location during the middle part of gestation (average date: 27th of June (± 3.5 days)) We measured snout-to-vent length, (SVL, $\text{mean} \pm \text{SE} = 62.4 \pm 4.3$ mm), and body mass when pregnant (BM_p , 5.14 ± 1.18 g) in the field and subsequently brought all females to a laboratory facility. Each lizard was individually housed in plastic terrarium under standardized conditions (Rutschmann *et al.*, 2015). Immediately after parturition, the females were weighed again (BM_{pp} , 3.14 ± 0.62 g). We recorded litter size and the length and mass of each offspring.

In lizard species, juvenile quality is often approximate by egg or neonate mass (Sinervo *et al.*, 1992; Bleu *et al.*, 2013). Neonate common lizards are born surrounded by a thin membrane and residual yolk that functions as an energetic reserve. However, as neonates emerge immediately after parturition (mainly at night) the measurement of eggs prior to hatching is not feasible. Therefore, we lack data on all of the energy spent for each juvenile. In addition, litters often contained dead neonates and unfertilized eggs, which desiccate very quickly, often before we find them. Thus, estimation of a female's total investment in her progeny is difficult. Eliminating these values from our sample would require using only litters consisting of all live offspring and rely solely on juvenile mass. However, this would considerably reduce our sample size and diminish the statistical power of our analyses. Hence, we decided not to estimate offspring quality using neonate mass. Rather we estimated an index of reproductive investment (RI) as the difference between the mass of a female when pregnant BM_p and her post-parturition mass BM_{pp} (Miles *et al.*, 2000). We derived the energy invested per offspring by dividing reproductive investment by the total litter size of each female, where litter size includes viable offspring, dead offspring and unfertilized eggs. Thus, the per capita investment in offspring is: $\text{Inv} = [\text{BM}_1 - \text{BM}_2] / \text{LS}$.

We obtained local weather conditions from the National Meteorological Agency (MeteoFrance, MF, <http://publitheque.meteo.fr>). We modified the weather data to match local temperatures as described in Rutschmann et al. (2015). In our analyses, we used the average of the daily maximal temperatures for June (T_{max6}). This temperature correspond to the mid-

gestation for our species and is the period in which temperatures have been demonstrated to be critical for juveniles' development in an oviparous snake (Lourdais *et al.*, 2004).

All statistical analyses were performed with R software (R Development Core Team, version 3.2.0). To study the variation on the trade-off between *Inv* and *LS* across the 11 populations we performed a three-step analysis. First, we estimated inter-population differences in the magnitude of the trade-off. To do so, we used mixed effect models where the random effect structure of the model was hierarchically extended from a model without inter-population differences (mod1), to a model where populations' trade-offs were allowed to vary in their slope (mod2). Details of the model selection are provided in supplementary material. Second, we assessed whether the position of each population along the thermal gradient influenced the intensity of local trade-offs. For every populations and for every years of capture, we extracted the slope coefficients " β " of a linear regression model between *Inv* and *LS* (i.e. the intensity of the trade-off). Then, for each population, we calculated the linear regression coefficient α between the different β and their corresponding T_{max6} which measure the flexibility of the trade-off to T_{max6} .

RESULTS

We found an overall negative relation between *Inv* and *LS* ($b = 0.011 \pm 0.001$; $t_{657} = -10.42$; $P < 0.001$). However, the random regression model showed significant inter-population differences in the intensity of the trade-off between reproductive investment (*Inv*) and litter size (*LS*) as the best model was a model where each population's trade-off could vary in its slope (AICc mod1 = -26553.1; AICc mod2 = -27131.9). Complete models and significance of fixed effects are provided in table A1 in the supplementary materials.

The analysis of trade-offs' intensity revealed a positive relation between β and T_{max6} for 8 populations out of 11 (all $\alpha > 0.0027$). This result suggests that the trade-off is less intense in warmer conditions. However, three populations exhibited a negative α (range between -0.0017 and -0.0021). Results are summarized in Table 1 and Fig.1. An additional linear model between α coefficients and population's average temperature confirmed that colder populations are less flexible and therefore cannot adjust the trade-off to inter-annual thermal conditions as well as warmer ones ($b = 0.0013 \pm 0.0002$; $t_9 = -4.44$; $P = 0.002$).

DISCUSSION

We have shown that local conditions influences the allocation of energy a female makes in current reproduction. Indeed, despite a general negative relationship observed between litter size and juvenile quality (i.e. trade-off), our results show significant differences in the intensity of the trade-offs between populations. For 8 populations out of 11, we detected a positive relation between the intensity of annual trade-offs (β) and $T_{\max 6}$. This result shows that the trade-offs, intense in poor years, is reduced when temperature increases and eventually collapses when environmental conditions become highly favorable. This result corroborates results from a previous experiment that revealed a positive correlation between environmental quality and the slope derived from the association of egg size and number (Ebert 1993). In ectotherms, both physiological performance and metabolic rate increase with ambient temperature (Dillon *et al.*, 2010). Higher efficiency in resource acquisition or a higher metabolic rate could facilitate females to accumulate sufficient energy reserves and to complete gestation, regardless of the number of fertilized eggs. Surprisingly, three populations showed a slightly negative relation between the magnitude of the trade-off (β) and the temperature. These populations are situated in the coldest locations of our survey and, even in warm years, climatic conditions are probably too restrictive to allow any attenuation of the trade-off. Our statistical analyses corroborate this hypothesis as they indicate that colder populations of the survey present a lack of flexibility in their trade-off (α close to zero).

Our analyses therefore suggest that the relaxing effect of warmer temperature observed at the intra-population level also exist at a larger scale: colder sites always impose restrictions in resources allocation, whereas at warmer sites females have the opportunity to maximize both litter size and litter quality as soon as conditions turn favorable. In the context of current climate change, the positive effect of temperature might have important consequences on common lizards' population dynamics. For example, Bestion *et al.* (2015) experimentally demonstrated a switch of demographic strategies in a warming experiment conducted in semi-natural conditions. Their results showed that warmer temperatures led to an accelerated pace of life (e.g. increased probability of pregnancy, larger juveniles mass), and would ultimately induce a rapid collapse in population densities by increasing adult mortality. In our study system, warmer temperatures relax the offspring number-quality trade-off, but paradoxically may strengthen other trade-offs (e.g. present vs. future reproduction or reproductive investment vs. future survival). Interactions between trade-offs are very well describe and play major roles in species evolution (Sinervo and Clobert 2008). For example,

environmentally dependent reorganization of trade-offs have been documented for spatial and temporal variation in food abundance (Reznick *et al.*, 2000; Ernande *et al.*, 2004a). Although we cannot confirm this hypothesis here, our results highlight the crucial importance to consider the major role of variation in environmental characteristics on the evolution of life-history traits in the current context of global change.

Figure 1: Influence of mid-gestation temperature on the intensity of the Trade-offs: β coefficients are calculated as the slope coefficients of a linear regression model between *Inv* and *LS*. First panel represent all β regrouped along the global thermal gradient. Other panels: for each population, we calculated α coefficient (linear regression coefficient) that measure the flexibility of β according to local *Tmax6*. We found positive coefficient for 8 populations out of 11, suggesting that constraints on the trade-off are weaker warmer years.

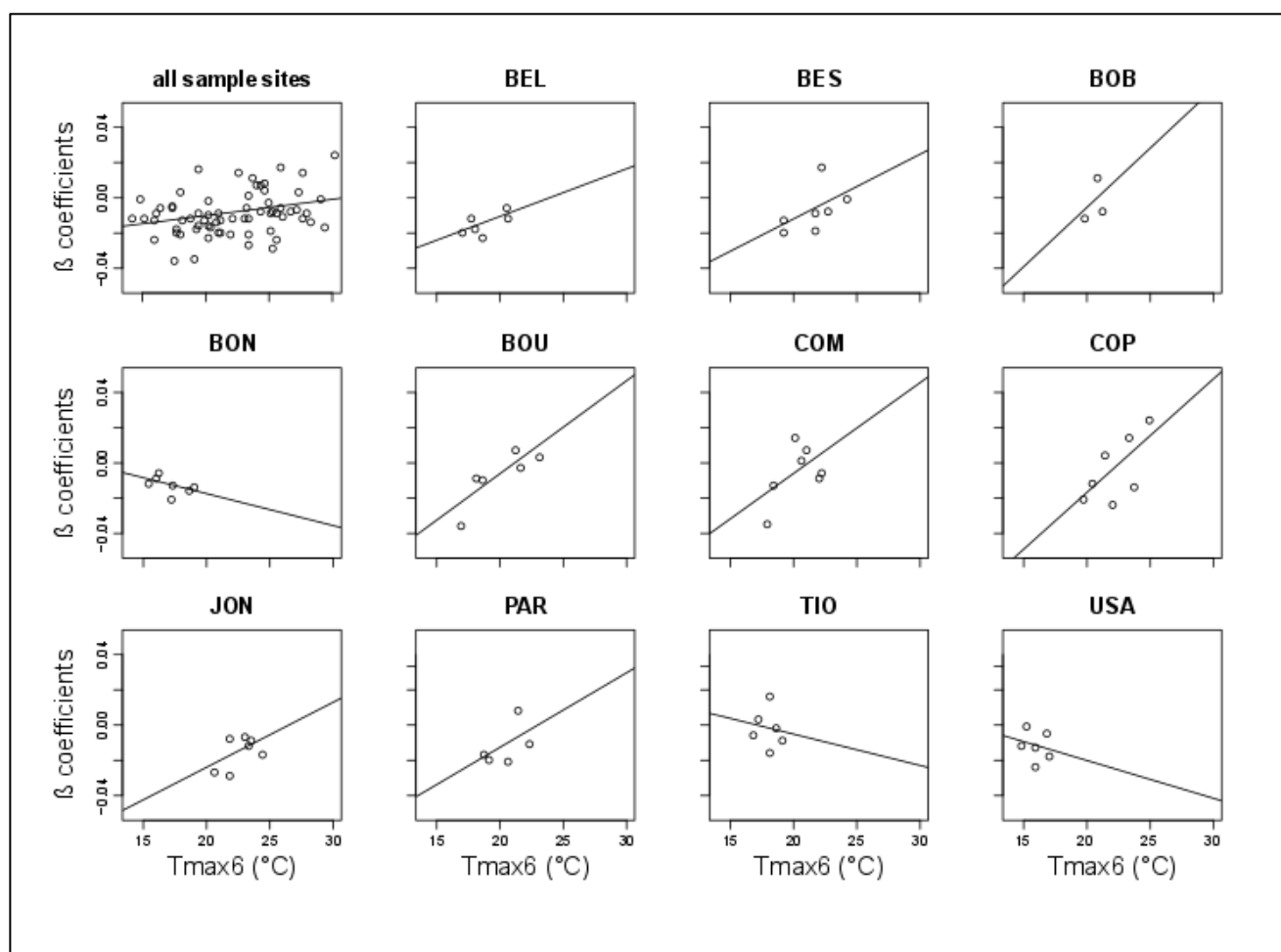


Table 1. Description of each population. Comparison between α coefficient and average mid-gestation temperatures on each population (Mean Tmax6). We also provide information about the number of sampling attempts and the Mountain massif.

Site	α	Effect of Tmax6	Mean Tmax6	Sampling attempts	Mountain Massif
BEL	0.0027	+	18.63 (± 0.45)	6	Vivaraïs
COP	0.0064	+	22.1 (1.87)	6	Vivaraïs
BES	0.0037	+	21.46 (1.82)	6	Vivaraïs
BOU	0.0052	+	19.82 (2.4)	6	Margeride
COM	0.0051	+	20.21 (1.66)	6	Margeride
PAR	0.0042	+	20.32 (1.52)	5	Margeride
BOB	0.0067	+	20.5 (0.72)	3	Velay
JON	0.0036	+	22.53 (1.29)	7	Velay
BON	-0.0018	-	17 (1.34)	7	Aubrac
TIO	-0.0017	-	17.88 (0.86)	5	Aubrac
USA	-0.0021	-	15.93 (0.86)	6	Aubrac

ANNEXE 1: Result of used mixed effect models analyses.

To check for inter-population differences in the intensity of the trade-off we used mixed effect models. The random effect structure of a model confronting *Inv* and *LS* was hierarchically extended from a model without inter-population differences (mod1), to a model where populations trade-offs were according to vary in their intercept (mod2) or in their intercept and slope (mod3). According to AICc differences, we retain the model that allowed inter populations differences in both elevation and slope (AICc mod1 = -17447.7; AICc mod2 = -17833.79; AICc mod3 = -17855.65). However, in the main manuscript, we only retained trade-offs' slope that is the only relevant parameter in our study. In the fixed effect part of the model, we controlled for the time spend in the laboratory facilities, that can affect female mass gain.

Table A1: Best linear mixed effects model summary table for energy invested by a female in each juveniles (*Inv*) versus Litter Size (*LS*) and time spend in the laboratory facilities (*Lab*), (parturition date - capture date). *LS* was nested within population (with population as a random effect). We added *Lab* as a fixed effect to control for the influence of breeding facilities temperatures and feeding rate on females' resource acquisition.

Predictor Variables	Estimate	Std. Error	d. f.	t-value	P-value
Intercept	0.507	0.01	6557	56.42	<0.0001
LS	-0.011	0.001	6557	-10.42	<0.0001
Lab	-0.006	0.0001	6557	-50.97	<0.0001
Random Effects	Std. Dev.	Corr.			
Fec.	0.003				
Residual	0.06	-0.7			

CHAPITRE 3 : Caractère adaptatif de la plasticité de la date de ponte.

Les résultats du chapitre 1 de cette thèse sont fondés sur une hypothèse forte : la norme de réaction observée pour chaque population repose sur des mécanismes plastiques. La plasticité phénotypique est cependant un mécanisme individuel. Les données du projet 'Global Change' ne nous permettant pas de valider cette hypothèse (absence de suivi individuel), j'ai utilisé la base de données de capture-recapture 'ROB' dans laquelle de nombreuses femelles sont capturées plusieurs fois au cours de leur vie. Pour chaque femelle capturée et mise en élevage au moins deux années consécutives, j'ai pu estimer une norme de réaction individuelle décrivant sa capacité à modifier sa date de mise bas en fonction des conditions thermiques rencontrées.

Chez les ectothermes, il est cependant parfois difficile d'interpréter la variation d'un phénotype en réaction à une composante thermique de l'environnement puisque la température affecte directement l'ensemble des processus biochimiques de l'individu. Ainsi, l'observation de plasticité phénotypique peut refléter à la fois l'expression d'une contrainte d'ordre physiologique ou l'expression d'une stratégie adaptative. Dans cette étude, j'ai voulu à la fois (1) comprendre comment la plasticité variait entre les individus, (2) appréhender le rôle du climat sur ces variations et (3) estimer l'avantage adaptatif que pouvait apporter cette plasticité.

Les résultats démontrent qu'il existe de la variation dans les normes de réactions des individus et que l'intensité de cette variation est directement liée aux conditions thermiques dont les individus font l'expérience. Ils démontrent également que bien que la plasticité de la date de mise bas procure un net avantage à l'échelle de la population, les femelles les plus plastiques produisent des juvéniles moins corpulents et grandissent moins entre deux événements de reproduction. Dans les années les plus chaudes (températures pour lesquelles la majeure partie de la variation interindividuelle apparaît), certains individus pourraient souffrir de l'accélération de leur métabolisme au cours de la gestation et leur réponse plastique deviendrait non-adaptative.

Article 3: Adaptive or not adaptive ? Ambiguous role of phenotypic plasticity in a lizard population.

For consideration in: Evolution, *under co-authors considerations*

Authors : Alexis Rutschmann¹, Murielle Richard¹, Manuel Massot², Donald B. Miles^{1,3}, & Jean Clobert¹

¹ CNRS, USR2936, Station d'Ecologie Expérimentale du CNRS à Moulis, Moulis, 09200 Moulis, France; ² CNRS/ENS/UPMC, UMR 7618, Laboratoire iEES Paris, Université Pierre et Marie Curie, 7 Quai St. Bernard, 75005, Paris, France; ³ Ohio University Department of Biological Sciences 131 Life Sciences Building, Athens Ohio.

Abstract :

Phenotypic plasticity, the response allowing a genotype to produce alternative phenotypes across multiple environment, is a mechanism by which individuals can cope with spatio-temporal heterogeneity of their environment. Different papers have demonstrated that inter-individual variation in plasticity exists and is sometime under strong selection. However, knowledge about ultimate mechanisms that drive variations between individuals in natural populations is still scarce. Here, using a long term survey of a population of common lizards, we tried to detect whether individual variations in parturition dates was connected to thermal environment. Moreover, we assessed whether the plastic response was adaptive in all environmental circumstances. Our results suggest that warmer conditions enhanced both the intensity of the plastic response and its variations between individuals. Besides, if earlier parturitions in warmer conditions are adaptive at the population level, we demonstrate that at the individual level, high levels of plasticity are not necessarily adaptive and decreases offspring quality and females growth.

Keywords : IxE, non-adaptive plasticity, environmental variations, common lizard

INTRODUCTION

Among the different mechanisms allowing populations to cope with rapid environmental changes, attention has mostly been focused on phenotypic plasticity (i.e. the ability for a given genotype to produce alternative phenotypes in response to different environmental conditions, fig 1a). It has theoretically and empirically been explored using a simple function, the reaction norm, which describes the phenotypic variation as a function of an environmental characteristic (fig1b). Considerable work has been done to estimate the adaptiveness of phenotypic plasticity, its costs and its genetic or molecular basis (Bradshaw, 1965; Gavrilets & Scheiner, 1993; Schlichting & Pigliucci, 1995; Sultan, 1995; Via *et al.*, 1995; de Jong, 2005). However, despite this strong interest, our knowledge about the causes and implications of variation of the reaction norms in natural populations is still scarce. Until recently, very few studies examined the environmental factors that drive variations between populations' or individuals' reaction norms (but see Sultan and Spencer 2002; Ernande *et al.* 2004b; Lind and Johansson 2007; Baythavong and Stanton 2010; Chevin *et al.* 2010; Porlier *et al.* 2012).

Long term studies, where repeated measures of similar phenotypes are made on numerous individuals across multiple environmental conditions, allowed us to progress significantly in the understanding of those variations. For example, they have been used to confirm that the response to environmental variations at the population-level is often associated with variations in plasticity at the individual-level (Nussey *et al.* 2005b; Charmantier *et al.* 2008; Fig 1c). Moreover, comparisons of several population of a same species demonstrated that plastic responses could also vary between populations (fig 1 d), pointing out a first mechanism by which individuals can vary in phenotypic plasticity (Nussey *et al.*, 2005a; Garant *et al.*, 2008). Theoretical models usually suggest that the degree of spatial heterogeneity in the environment is a major driver of the evolution of phenotypic plasticity, and that it could influence both the average level of the plastic response (i.e. the elevation of the reaction norm fig 1 e) or its magnitude (the slope of the reaction norm, fig 1f; Ghalambor *et al.* 2007b; Nussey *et al.* 2007; Chevin *et al.* 2010). These predictions have since been confirmed using both experimental studies and long term surveys (Lind & Johansson, 2007; Porlier *et al.*, 2012; Rutschmann *et al.*, 2015). Finally, analyses of long-term surveys have been used to highlight variations in phenotypic plasticity between individuals within a same population (hereafter termed as IxE), some of the observed individuals being more plastic in their phenotype than others (Réale *et al.*, 2003a; Brommer *et al.*, 2008b;

Dingemanse *et al.*, 2012a). This result is not really surprising as plasticity is known to be a heritable and selected trait (Scheiner, 1993; Nussey *et al.*, 2005b; Pelletier *et al.*, 2007; Charmantier *et al.*, 2008). Several mechanisms can lead to different patterns of IxE, such as differences in ecological conditions experienced by individuals across their life time or variations in individual quality (see Box1, Fig1g; h.). In the current context of global change, assessing the causes and consequences of variation in plasticity is a crucial challenge as it would help to estimate the potential genetic variance that selection can act upon and therefore to determine the evolutionary potential of a population (Pigliucci, 2001; Chevin *et al.*, 2010).

During the past decades, many studies have reported rapid changes in average phenotypes, often associated to changing climatic conditions or to human activities (e.g. changes in phenology (Root *et al.*, 2003; Parmesan, 2006), morphology (Sheridan & Bickford, 2011), physiology (Dillon *et al.*, 2010) or coloration (Zeuss *et al.*, 2014). Even if some recent examples showed that fast adaptive genetic response can emerge due to environmental variations (Bradshaw *et al.*, 2006; Gienapp *et al.*, 2008) , most of them may be based on plastic mechanisms. As a consequence, there has been an increase of interest in phenotypic plasticity, and more specifically for life history traits responding to variations in ambient temperature, which is a crucial environmental factor for every organism. Moreover, ambient temperatures are of major importance for terrestrial ectotherms as they affect phenotypes by directly acting on biochemical processes. To reduce these environmental effects, ectotherms can partially buffer stressful changes through behavioral modifications (e.g. basking activity) and thus maintain a "preferred" body temperature in various thermal conditions (Huey *et al.*, 2003; Kearney *et al.*, 2009; Sinervo *et al.*, 2010; Clusella-Trullas *et al.*, 2011). As a consequence, an observed phenotype may be the result of biochemical constraints or the expression of behavioral plasticity or, more likely, the interaction of both mechanisms. Observed I x E can in turn be the direct result of inter-individual variations in biochemical processes, or the result of inter-individual differences in behavioral plasticity, or the interaction of inter-individual variations in both mechanisms. In this context, it is particularly complex to assess whether phenological plasticity is adaptive or not and thus to assess how phenological plasticity will act on the evolution of ectotherms populations.

In this study, we focused on a small lacertidea, *Zootoca vivipara*, to explore inter-individual variance in phenotypic plasticity for parturition date (PD). Recently, many studies have been focusing on such phenological traits (e.g. laying date, hibernation, migration...) that strongly respond to temperature and are classically correlated to fitness (Chuine, 2010). These

relationship are well documented as they are often varying along the individual lifetime according to the thermal conditions encountered (Parmesan & Yohe, 2003; Brommer *et al.*, 2005). As a viviparous species, the common lizard embryogenesis is completed in the female's abdomen and last on average two and a half months. Although the effect of climatic conditions on body temperatures may be buffered by compensatory responses of basking behaviour, thermal environment has important effects on the duration of the embryogenesis. Indeed previous studies have highlighted earlier parturition dates when temperature during mid-gestation is warmer (Chamaille-Jammes *et al.*, 2006; Lepetz *et al.*, 2009; Le Galliard *et al.*, 2010). It has also been demonstrated differences in environmental conditions lead to among-populations variation in reaction norms (Rutschmann *et al.*, 2015). However, variations in reaction norms between individuals from a single population have never been explored yet in this species.

To estimate the evolutionary importance of inter-individual differences in phenological plasticity, we tested whether (i) the already described advancement in parturition date was, at least partially, the results of individuals' plastic responses and (ii) if there were any evidences of inter-individual differences in plasticity. Then (iii), we assessed if the individual plasticity we observed provided any selective advantage to the pregnant females or their juveniles. Finally (iv), we checked if the variation in the magnitude of the reaction norms was constant along the environmental range experienced by the population

METHODS

Zootoca vivipara

The common Lizard *Z. vivipara* is a widely distributed Lacertidae. Most of the European and Asian populations are known to be ovoviparous except for the populations inhabiting south or extreme-east margins of the distribution, which are oviparous (Surget-Groba *et al.*, 2001). In our study area, adult males emerge from hibernation in late April, just before yearlings and adult females. Mating occurs early in May, immediately after the females' emergence. Egg fertilization is initiated just after copulation and gestation occurs between the beginning of May and the middle of July (at the latest early August). After parturition (average = 29th of July, $\pm$ 9 days), individuals accumulate energy reserves until hibernation which starts in October. A complete life cycle is provided in Bleu *et al.* (2013). Females rarely live longer than 6 years, start to reproduce at 2 years old and live between 1 and 5 events of reproduction.

Population monitoring and breeding conditions

To assess whether or not observed relation between June temperature and parturition date (PD) rely on plastic mechanisms, we used a long-term study (LTS), sampled from 2003 to 2015 nearby the Mont Lozère (Southern France, 44°30'N 3°45'S, 1420m altitude). We sampled LTS population in mid- June using mark-recapture techniques. Each individual was marked at first capture with a unique combination of toe-clipping (or at birth when birth occurred in the laboratory). Toe-clipping neither affects the probability of recapture or survival (Massot *et al.*, 1992). After capture, pregnant females were measured (snout to vent length (SVL), mean = 60.1 ± 4.9 mm), weighed (body mass before parturition: $BM_{bp} = 4.76 \pm 1.08$ g) and brought to the laboratory until parturition. There, they were individually housed in terraria (11x18x11 cm) until parturition (Massot & Clobert, 2000a). Females were exposed to the local prevailing photoperiod, but provided with access to heat for 6 hours per day (twice 3 hours, one in the morning and one in the afternoon, the average temperature in the breeding was of 25.19°C (s.d. = 2.31). Immediately after parturition, females are weighed (post-parturition body mass BM_{pp}) and juveniles are described. Each female was released with its juveniles three days after parturition at its exact capture location. Because sampling planning varied between years according to local weather conditions, parturition dates were statistically corrected by the date of capture.

We will focus here on 180 females (within the 398 we captured between 2003 and 2015) that reproduced consecutively at least twice in our breeding. We will hereafter use the term of "*cohort*" for females which reproduced on the same two consecutive years (e.g. cohort [2004; 2005] or [2006; 2007]).

Climatic data

Daily air temperatures were obtained from the national French meteorological agency data-base (MeteoFrance, <http://publitheque.meteo.fr>). Temperature data were recorded at the Mont-Aigoual meteorological station (Chamaille-Jammes *et al.*, 2006). We used the average of the maximal daily temperatures of June (T_{max6}) as this value has been shown to be a relevant predictor of thermal conditions during gestation (Chamaille-Jammes *et al.*, 2006; Lepetz *et al.*, 2009). Moreover, Lourdaïs *et al.* (2004) statistically demonstrated that embryos development is the most sensitive to temperature in the middle part of the gestation in an

oviparous snake (*Vipera aspis*). For the common lizard, this period of gestation correspond to June (Bleu *et al.*, 2013), just before the period of captures.

Population and individual response to temperature

To study the average response of the population to variations in temperature during the mid-gestation period, we first estimated the relationship between parturition date (PD) and T_{max6} using a classical linear model (lm procedure of R) and including age and SVL (snout-vent length) as fixed effects.

Then, we assessed whether the relationship between temperature at mid-gestation and PD, at the population level, was based on individual phenotypic plasticity and if inter-individual differences in the responses to environmental variations existed (i.e. genetic diversity in phenotypic plasticity). Using all the females from our database (n=398), we sequentially compared four mixed effects models. The random effects structure of the model was hierarchically extended to test if there were 1/ no inter-individual differences; 2/ any inter-individual differences in PD mean values of (-I-, random intercept model); 3/ inter-individual differences in the response to environmental variation (-IxE-, random slope model); and 4/ inter-individual differences in both of them (-I- + -IxE-, random intercept and slope model). The following models were tested:

<i>Model 1: Minimal model</i>	$PD \sim \text{fixed effects}$
<i>Model 2: Random intercept</i>	$PD \sim \text{fixed effects, random} = \text{Individual}$
<i>Model 3: Random slope</i>	$PD \sim \text{fixed effects, random} = Tm6 * \text{Individual}$
<i>Model 4: Random intercept and slope</i>	
$PD \sim \text{fixed effects, random} = \text{Individual} + Tm6 * \text{Individual}$	

Once the optimal structure of the random component had been selected (Likelihood ratio Test, Zuur *et al.* 2009), we determined the fixed part of the model (SVL, age and $Tm6$) using backward selection (threshold of significance < 0.05). In the Model 4, the significance of the correlation between intercept and slope parameters was also tested by comparing the Model 4 and a model in which the covariance between the parameters was fixed to zero (lmer procedure of R, ade4 package).

Individual plastic response, individual characteristics and environmental variations

To explain the intra-individual variations observed in the previous analysis, we estimated for every lizard the strength of its phenotypic plasticity for PD. To do so, we calculated the within individual variation in PD (Δ_{PD}) as the difference between PD in 2nd reproduction and PD in the 1st reproduction ($\Delta_{PD} = PD_2 - PD_1$; Porlier et al. 2012). Then, we tested whether this coefficient was affected by the following individual characteristics: growth rate (difference in size between the two consecutive reproductive seasons, $\Delta_{SVL} = SVL$ at 2nd reproduction - SVL at 1st reproduction), age (the female age at the first observed reproductive event) and the difference in fecundity ($\Delta_{FEC} = \text{fecundity at 2nd reproduction} - \text{fecundity at 1st reproduction}$). We also included in our analyses differences in environmental conditions during the mid-gestation period ($\Delta_{T_{max6}} = T_{max6}$ during the second reproduction - T_{max6} during the first reproduction). The complete model was: $\Delta_{PD} \sim \Delta_{SVL} * \Delta_{FEC} * \Delta_{T_{max6}}$. We assessed the significance of first order interactions and simple main effects using backward elimination of non-significant terms (threshold of significance < 0.05).

Impact of plasticity on individual fitness.

To evaluate whether phenotypic plasticity was adaptive (i.e. helped females to maintain or even improved their fitness), we examined its impact on three components of females' fitness. We only focused on the reproductive contribution to fitness since we did not have enough data to evaluate its impact on survival. First, we estimated the impact of Δ_{PD} on the investment of female in reproduction (averaged post gestation loss of weight due to parturition: $RI = BM_{bp} - BM_{ap}$) using classical linear model (lm function in R). We controlled for the averaged SVL and averaged fecundity, implementing them as fixed effects in the model. Second, the direct effect of plasticity on reproduction by testing the impact of Δ_{PD} on the litter size (LS). SVL was again used as a cofactor in the model. Finally, we estimated the effect of Δ_{PD} on the quality of the juveniles (defined here as the mean mass of the two clutches (m_{Juv})) as this value influences juvenile survival until, during and after hibernation (Bestion *et al.*, 2015). We controlled for the number of juveniles per clutch and the female size. For all three models, we assessed the significance of first order interactions and simple main effects using backward elimination of non-significant terms (threshold of significance < 0.05 , Zuur *et al.*, 2009).

Inter-cohort variations in individual response to temperature

Finally, we explored if the amount of variation between the slopes of the reaction norms linking PD and temperature was varying along the thermal gradient experienced by the population, with some cohorts potentially exhibiting important differences between females' reaction norms slopes and some showing weak differences. First, we calculated the variance in Δ_{PD} within every cohort ($V\Delta_{PD}$ = intra-cohort Variance in Δ_{PD}). Then, we tested with a classical linear model whether this variance was correlated with experienced environment in the following model: $V\Delta_{PD} \sim Tm6_{(1)} + Tm6_{(2)} + Tm6_{(1)} * Tm6_{(2)}$ where $Tm6_{(1)}$ represents conditions during mid-gestation at first reproduction and , $Tm6_{(2)}$ conditions during mid-gestation at second reproduction, the interaction $Tm6_{(1)}$ by $Tm6_{(2)}$ represents the divergence in conditions between the two consecutive reproductive seasons.

RESULTS

Population response to temperature reflect individual plasticity

Parturition Date (PD) was influenced by local mid-gestation temperatures with females giving birth earlier in warmer years (T_{max6} Estimate: -3.37 ± 0.05 ; $t_{394} = -18.9$; $P < 0.0001$). The random regression models showed significant inter-individual differences in both the mean and the slope of individual responses to environmental variations (AICc minimal model = 2395.0; AICc random intercept model = 2385.2; AICc random slope model = 2382.1; AICc random intercept and random slope model = 2379.4), suggesting that the population response in PD was based on individual plasticity. Controlling for covariance between elevation and slope did not significantly improve our models. Selection of fixed effects (T_{max6} , SVL, Age, Time spent in the laboratory) led to a final model where only temperature during mid-gestation and females length affect significantly PD. The model is summarized in Table1.

Link between variations in plasticity and individual or environmental characteristics

Results of the analysis of individual plasticity revealed a negative effect of $\Delta_{T_{max6}}$ on Δ_{PD} (Estimates: -1.95 ± 0.17 ; $t_{128} = -13.27$; $P < 0.0001$) and of Δ_{SVL} on Δ_{PD} (Estimate: -1.01 ± 0.20 ; $t_{250} = -5.03$; $P < 0.0001$). However, the difference of litter sizes between successive reproduction did not influence the strength of individual plasticity (Estimate: 0.001 ± 0.29 ; $t_{250} = 0.006$; $P = 0.99$). This results suggest that Δ_{PD} increases when differences in mid-gestation conditions are important, with a significant delay when the second year was poor

and a significant advancement when the second year was warmer. They also suggest that females have to deal with a trade-off between their own growth and their plasticity as an important growth between two reproductive seasons (large Δ_{SVL}) prevent females to adjust their PD (weak Δ_{PD}).

Impact of Plasticity on fitness

We do not found any effect of the strength of individual plasticity (Δ_{PD}) on litter size (*LS*, *Estimate*: 0.03 ± 0.022 ; $t_{104} = 1.044$; $P = 0.11$). However, plasticity was almost correlated with the *mJuv* (*Estimate*: -0.4 ± 0.21 ; $t_{142} = -1.84$; $P = 0.06$) and correlated with *RI* (*Estimate*: -0.02 ± 0.008 ; $t_{104} = -2.17$; $P = 0.03$). Models results are summarized in Table 2 and Fig.1.

Effect of temperature on inter-individual variation in plastic response.

The analyses of divergence between patterns of plasticity within cohorts ($V\Delta_{PD}$) showed that both *Tm6₍₁₎* (*Estimate*: -2.61 ± 11.63 ; $t_{13} = -2.375$; $P = 0.034$) and *Tm6₍₂₎* (*Estimate*: -24.83 ± 11.49 ; $t_{13} = -2.162$; $P = 0.049$) decreased the divergence between females reaction norms in a given cohort. However, these effects are affected by a significant interaction between *Tm6₍₁₎* and *Tm6₍₂₎* (*Estimate*: 1.98 ± 0.8 ; $t_{13} = -2.421$; $P = 0.031$). This result suggests that the more mid-gestation conditions between reproductive events are different, the more the heterogeneity between reaction norms in a given cohort will be important. Moreover, we detected a significant relation between the intensity of plasticity and the temperature (*Estimate*: -0.71 ± 0.31 ; $t_{129} = -2.259$; $P = 0.025$). This result suggests that females are more plastic in warmer conditions as they are more likely to advance when temperature is high).

DISCUSSION

Phenological responses to environmental conditions have been observed in many species and can vary between populations and individuals. In this study, we explored the impact of mid-gestation temperatures on parturition timing in a highland population of the common lizard (*Zootoca vivipara*) and highlighted inter-individual differences in phenotypic plasticity. However, despite a significant benefit to speed up the gestation in warmer years, we showed that high levels of plasticity had a negative effect on juveniles' quality and on females' post-

reproductive condition. Moreover, our results indicated that the level of plasticity in parturition date and its variance in the population ($V\Delta_{PD}$) varied along the thermal gradient experienced by the population.

At the population-level, PD was generally earlier when females experienced warmer temperatures during mid-gestation. This pattern is consistent with previous findings by Chamaillé-James *et al.* (2006) and Le Galliard *et al.* (2010) who already demonstrated, working on the same population, that females had specific thermal requirements during this specific period (i.e. June). It is also consistent with other long term studies that have reported significant relationships between climatic conditions and PD in several wild reptile populations (aspis viper (*Vipera aspis*; Lourdais *et al.* 2004), snow skink (*Niveascincus ocellatus*; Cadby *et al.* 2010), sand lizard (*Lacerta agilis*; Ljungström *et al.* 2015), and sleepy lizard (*Tiliqua rugosa*, Bull and Burzacott 2002). Our results take our knowledge on the common lizard one step further as they demonstrate that the trend observed at the population-level is induced by individual phenotypic plasticity. Moreover, they suggest that females varied significantly in their responses to mid-gestation temperature. This observation is now a classic one as inter-individual variations in phenotypic plasticity has been observed among different populations of different species (Przybylo *et al.*, 2000; Brommer *et al.*, 2005; Nussey *et al.*, 2005a; Charmantier *et al.*, 2008; Piché *et al.*, 2008; Klepsatel *et al.*, 2013). The occurrence of these variations suggests that natural selection has not yet depleted the pool of available variance for this trait. But, due to changing climatic conditions, we may expect to see in a near future micro-evolutionary responses to selective pressures acting on individual plasticity (Nussey *et al.*, 2005a; Gienapp *et al.*, 2008; Husby *et al.*, 2011).

Individual plasticity and fitness

Plasticity is predicted to be adaptive when the production of alternative phenotypes helps individuals to perform better across multiple environments (Pigliucci, 2001). Several studies have already demonstrated in different Squamate species that plasticity in parturition date is adaptive. Indeed, laying earlier in warmer years improve the fitness of both the mothers and their juveniles, as they benefit from a longer period before hibernation. On the opposite, to delay the gestation when conditions are not optimal may allow females to avoid giving birth in an unfavorable environment (Chamaillé-Jammes *et al.*, 2006; Ljungström *et al.*, 2015). Despite this significant pattern at the population level, we found no clear

association between higher phenotypic plasticity and higher fitness at the individual level. First, we found no direct link between plasticity in PD and litter size. This result is however not surprising as the common lizard is a capitalizing species, "deciding" on the number of eggs to be produced before mating and thus before the impact of mid-gestation conditions (Bleu *et al.*, 2011). We also pointed out a significant and negative relationship between females' plasticity and their reproductive investment and a marginally significant and negative effect of plasticity on neonates' quality. In other words, high levels of plasticity reduce litter's quality and reduce the energetic investment of the female in its clutch.

In a recent paper, Chevin & Lande advocate that negative patterns of plasticity does not necessarily reflect non-adaptive plasticity (Chevin & Lande, 2015). Apparently maladaptive plasticity may actually reflect an adaptive response to environmental or physiological parameters neglected in the analysis. If it is hard to discard this hypothesis, our results suggest however that the magnitude of the plastic response is negatively correlated to growth between two reproductive seasons. That is, there is a trade-off between growth rate and gestation duration for the allocation of energetic resources. For Squamate species, growth is continuous through life, and directly influence the litter size by increasing space availability in the abdomen (Shine, 2005). Restricting growth between two reproductive seasons therefore challenge future reproductions (Harshman & Zera, 2007). As a consequence, the plastic response of PD seems adaptive up to a certain limit but beyond this threshold, compromise emerges between plasticity and life history traits (e.g. juvenile quality, future reproduction...), leading to a significant loss in fitness.

Inter-individual variation in plasticity and thermal conditions

Interestingly, our results show that plastic responses are not constant along the thermal gradient. Indeed, they suggest that, in warmer environments, females not only are more plastic but also present more variations between their reaction norms. The variation between individuals' reaction norms ($V\Delta_{PD}$) is weak when females experience cool temperatures as, under such circumstances, conditions are restrictive for all individuals and differences in the reaction norms are small. However, both the intensity of the reaction norm and its variance between individuals ($V\Delta_{PD}$) increase when mid-gestation temperatures increase. Consequences of high temperatures on ectotherms are manifold. For example, warmer environments enhance the metabolic rate that in turn increase energetic needs (Sinervo *et al.*,

2010). Paradoxically, they also induce a restriction of lizard activity opportunities by increasing the length of the period during which environmental temperatures dangerously increase the body temperature (T_b). Consequently, warmer temperatures will affect the opportunities of daily energetic intake (Kearney *et al.*, 2009; Sinervo *et al.*, 2010; Vickers *et al.*, 2011). Obviously, common lizards can partly buffer the impact of environmental temperatures, adjusting their body temperature (T_b) via behavioral thermoregulation (Huey *et al.*, 2012). For example, they can find refuges in cooler areas of their habitat when temperature augment and take benefits of good thermoregulation spots when temperature declines (at the beginning or the end of the day, cloudy periods of the day...). In this context, females that do not benefit from a thermally contrasted home range will suffer from high temperatures. Consequently, they will accelerate their gestation due to increased metabolic rate, exhibit sharper reaction norms and ultimately produce weaker juveniles and exhibit a weak inter-season growth (Dillon *et al.*, 2010; Bestion *et al.*, 2015). On the opposite, females that can buffer the effect of heat will maintain a good resource acquisition rate and partly buffer the impact of war temperature on embryogenesis speed.

CONCLUSIONS

In ectotherms, plastic responses to temperature are always difficult to interpret as all physiological processes are constraint by environmental conditions. They can either reflect an adaptive response to temperature or a physiological constraint. Our results (i) confirm that the relationship between parturition dates (PD) and mid-gestation temperatures already observed at a population level rely on individual plasticity; (ii) provide evidence that the plastic response varies between individuals, according to both environmental changes and individual characteristics; and (iii) suggest that the advantage of plasticity at the population level is not parallel at the individual level. Our study thus emphasizes the need to consider individual and environmental characteristics when exploring inter-individual differences in phenotypic plasticity, but also their variation through time. We also suggest that, when studying variations in plasticity, one should check its fitness' implications as it can be the resultant of physiological processes, compensatory mechanisms and/or adaptive phenotypic plasticity.

Figure 1. Environmental impact on the reaction norms. Individual reaction norms are represented in black while population reaction norms are represented in red. a) Annual variations of the phenotype due to phenotypic plasticity. (b) Representation of the plastic response via its reaction norm: each phenotype is related to the environmental conditions it has been expressed in. (c) Because it is not common to sample individuals in all the environmental conditions their populations can experience, we need to sum individuals reaction norms to approximate the population reaction norm. (d) Variation of the reaction norm also exists at the inter-population level. Different scenarios of reaction norms variation. (e) Absence of inter-individual variation for the reaction norm in a given population. (f) Between individual variation in the reaction norm. Artefactual variations between individuals due to (g) a non linear reaction norm or (h) the expression of constraint on the plastic response in particular environmental conditions.

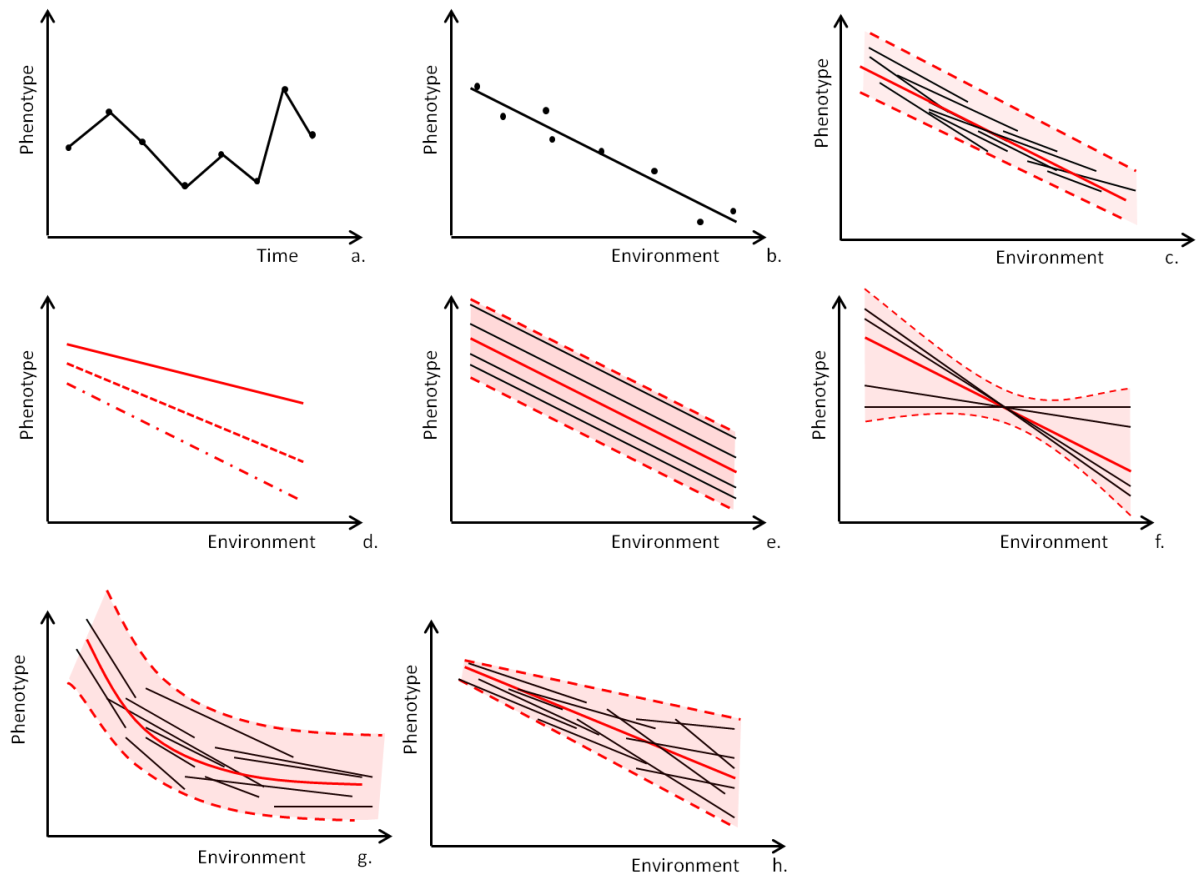


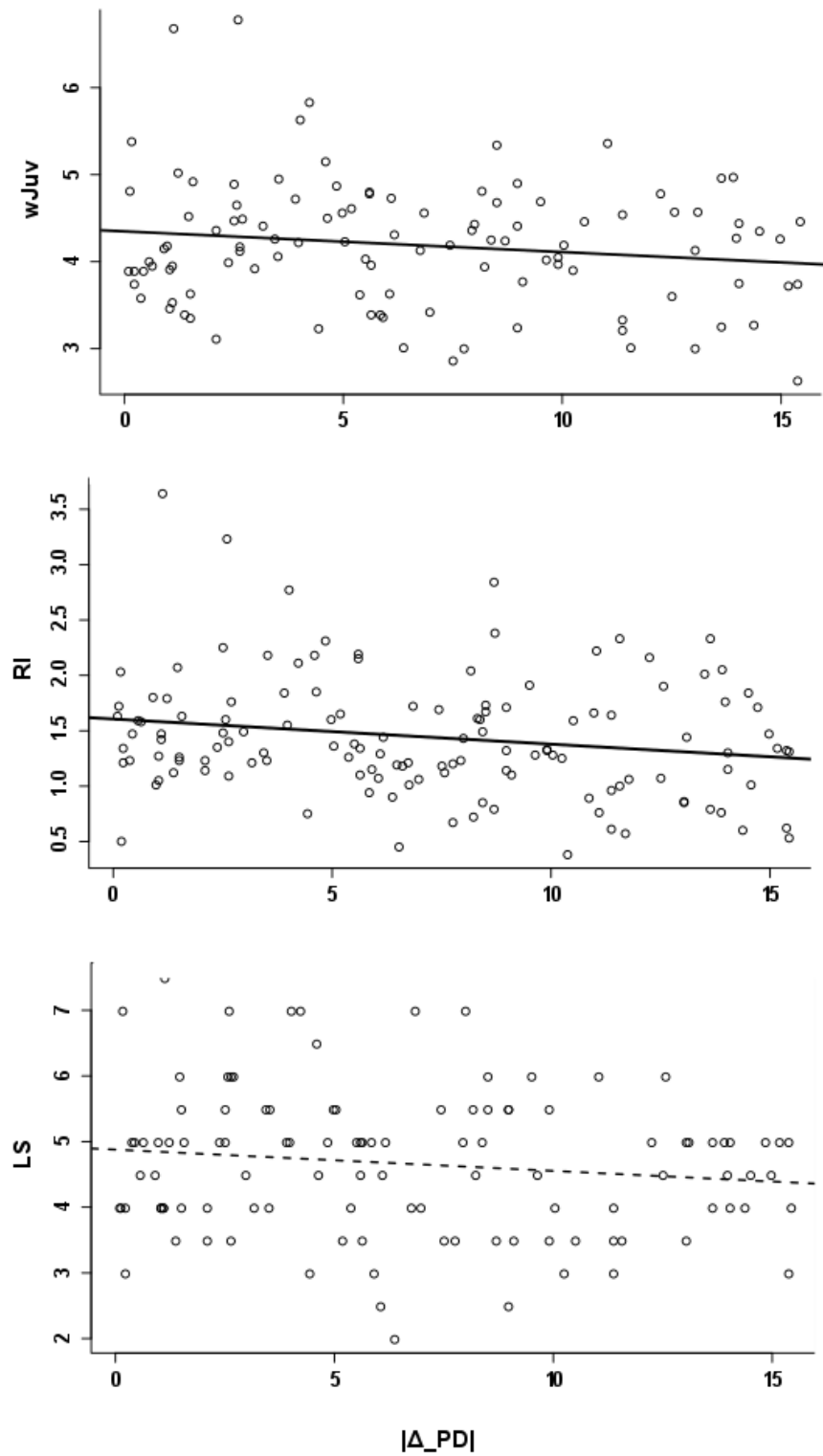
Table 1. Best linear mixed effects model summary table for parturition date express by a female according to SVL and T_{max6} . Individuals were nested within environmental temperature (as random effects) to detect inter-individual differences in their response to temperature (i.e. plasticity).

Predictor Variables	Estimate	Std. Error	d.f.	t-value	P-Value
Intercept	210.1*	0.25	214	-1.21	0.23
SVL	-0.31	0.057	214	-6.91	<0.0001
T_{max6}	-3.57	0.022	214	-19.18	<0.0001
Random effects	Std. Dev.	Corr			
Individual (intercept)	1.8				
Individual: T_{max6} (slope)	1.02	0.32			

Table 2. Summary of the effects of plasticity on reproduction. We estimated the impact of Δ_{PD} on (1) the investment of females in their reproduction calculated as the post gestation loss of weight due to parturition (*Inv*), (2) the litter size (*LS*) and (3) the quality of their juveniles (*mJuv*). Significant effects are in bright

	Estimate	Std. Error	d.f.	t-value	P-Value
Juvenile averaged weight (<i>wJuv</i>)					
Intercept	99.11	20.84	-	4.7	<0.0001
Plasticity	-0.4	0.21	104	-1.84	0.06
Fecundity	-3.76	0.93	104	-4.02	0.0001
SVL	1.67	0.37	104	4.48	<0.0001
Reproductive investment (<i>Inv</i>)					
Intercept	-4.07	0.72	-	-5.65	<0.0001
Plasticity	-0.02	0.008	104	0.16	0.02
Fecundity	0.04	0.03	104	-2.31	0.15
SVL	0.08	0.01	104	6.92	<0.0001
Litter Size (<i>LS</i>)					
Intercept	-9.91	1.93	-	-5.13	<0.0001
Plasticity	-0.018	0.018	107	-1.003	0.32
SVL	0.21	0.012	107	7.75	<0.0001

Figure 2: Phenotypic plasticity and reproduction: (a) Females that are less plastic produce larger juveniles. (b) Larger plasticity is associated with weak investment in reproduction. (c) There was no relation between plasticity and Fecundity .



Box 1: Factors influencing inter-individuals variation in phenotypic plasticity.

Several hypotheses could explain the presence of individual heterogeneity in phenotypic plasticity into a given population.

1. Differences in the ecological conditions experienced by individuals

Firstly, if the space or time scale of the environmental heterogeneity is larger than the life time of the individuals, every individual will face only a part of the historic conditions with which the population is confronted (Nussey *et al.*, 2007). If the population reaction norm is not linear (fig1 e), the amplitude of the phenotypical change should not be proportional to the amplitude of the environmental change and some inter-individual differences may emerge as a function of the response to the environmental gradient (Dingemanse *et al.*, 2012b). Moreover, stressful conditions might play a role of major importance in the expression of within-population plastic variance (Ghalambor *et al.*, 2007b). Indeed, they can reveal cryptic phenotypes that would not be expressed under classical environmental conditions but that will only appear in stressful years, increasing the variability associated with the expression of phenotypic plasticity (Rutherford, 2000, 2003; Ruden *et al.*, 2003; Fig1.f).

2. Interactions between plastic traits and other metabolic traits.

This would be the case if the observed plastic response is involved in a trade-off with another trait or another plastic relation, themselves fluctuating according to changes in other ecological parameters. Vrtílek & Reichard (2014) recently demonstrated in a small African fish that females adaptively influence the trade-off between size and mass of their clutch to match environmental conditions. In fact, this response has been demonstrated to be the direct consequence of the way females modulate their energy allocation to growth and reproduction. Another good example is provided by Nussey *et al.* (2005 b) who demonstrated that the females' ability to produce heavier offspring in warmer year was restricted by population density and decreased in years with higher level of competition.

3. Classical mechanisms maintaining inter-individual variations in the phenotype.

Heterogeneity could be maintained by negative frequency-dependant selection (Wolf *et al.*, 2007) or by the recruitment of new individuals in the population that do not share the native phenotype (Lenormand, 2002).

CHAPITRE 4: Mise en place et description du pédigrée.

1. Préambule

L'étude du pédigrée, de l'héritabilité de plusieurs traits phénotypiques (dispersion, date de ponte) ainsi que des corrélations génétiques existant entre certains traits (patron de mélanisation, dispersion, sexe, ...) aurait dû être le sujet central de cette thèse. Cependant, le temps nécessaire pour mettre en place le pédigrée a été sous estimé et sa création a largement dépassé les 3 à 6 mois qui avaient été prévus au moment d'établir le calendrier prévisionnel de la thèse. Ce travail qui aura occupé une grande partie de mon temps n'apparaît ici que sous la forme d'un résumé statistique du pédigrée et d'une ébauche d'article. Il nous offre cependant de très bonnes perspectives de travail qui se poursuivront, je l'espère, après ma thèse.

2. Définitions et concepts de génétique quantitative

La génétique quantitative peut-être définie comme l'étude génétique de traits complexes (ou quantitatifs continus, multigéniques). Comme leur nom l'indique ces traits sont déterminés par un grand nombre de gènes, parfois dispersés dans l'ensemble du génome. Parmi les plus étudiés en biologie évolutive, on trouve des traits morphologiques (comme la longueur de tarse pour les oiseaux), des traits phénologiques (date de mise bas, date d'hibernation), des traits d'histoire de vie (taille de ponte, dispersion) ou des traits comportementaux (agressivité, exploration). La génétique quantitative cherche plus précisément à comprendre l'origine des différences entre les phénotypes des individus qui composent une population en séparant la part d'influence génétique de la part environnementale. En d'autres termes, elle cherche à décomposer, à l'échelle de la population ou de l'espèce, la variance phénotypique (**VP**) existante en une variance génétique (**VG**) et une variance environnementale (**VE**; voir encadré XI pour plus de précisions). Déterminer et quantifier cette variance génétique au sein d'une population ou d'une espèce est primordial puisqu'elle permet de répondre à une multitude de questions en biologie évolutive (concernant par exemple l'héritabilité de traits morphologiques ou comportementaux, la contrainte

Encadré XI : Principe de la génétique quantitative: décomposition de la variance phénotype et calcul de l'héritabilité

Le principe de la génétique quantitative repose sur la décomposition de la variance phénotypique des traits polygéniques continus au sein d'une population et dans un environnement donné. La variance phénotypique se décompose de la manière suivante :

$$VP = VG + VE + VR$$

Où **VP** représente la variance phénotypique du trait d'intérêt dans la population, **VG** la variance due aux effets génétiques et **VE** la variance due à l'effet de l'environnement. Dans la définition classique de **VP**, la corrélation entre les génotypes des individus et les variations environnementales est supposée nulle ($Cov(G,E) = 0$), ce qui implique que les gènes s'expriment de la même manière à travers les différents environnements et que l'on ignore la plasticité phénotypique. Dans les modèles statistiques, la variance due aux effets environnementaux est souvent assimilée à la variance résiduelle du modèle **VR**.

La variance due aux effets génétiques **VG** peut elle aussi être également séparée en plusieurs composantes:

$$VG = VA + VD + VI$$

Où **VA** représente la variance génétique additive (ou variance due à la somme des effets simples de chaque locus déterminant le trait d'intérêt), **VD** la variance due aux effets de dominance entre allèles et **VI** la variance due aux interactions entre gènes (ou effets d'épistasie). En général, seul **VA** est considérée, les autres composantes étant trop complexes à déterminer.

Cette décomposition de la variance permet de calculer (1) l'Héritabilité au sens large (H^2) qui représente le degré de variation phénotypique expliquée par les gènes de l'individu (**VG**) et (2) l'héritabilité au sens strict (h^2) qui représente le degré de variation phénotypique expliqué uniquement par les effets additifs des gènes transmis à la descendance (**VA**).

$$(1) H^2 = VG / VP$$

$$(2) h^2 = VA / VP$$

génétique pouvant exister dans le cadre des compromis entre traits d'histoire de vie, l'importance des effets maternels, l'étude de la sénescence, l'étude de la sélection sexuelle, l'impact des changements globaux ou de la consanguinité sur la dynamique des populations,...).

De nombreuses méthodes permettent d'estimer la part génétique qui intervient dans la formation d'un phénotype et il est possible d'en résumer les fondements par l'hypothèse suivante: "*the degree of phenotypic similarity between relatives should reflect the genetic control of that trait*" (Kruuk, Loeske E. B. et al. 2014). Le degré de similarité phénotypique entre individus apparentés est donc au cœur des méthodes de génétique quantitative puisqu'il permet d'accéder indirectement à l'influence du génome sur le phénotype. Car, s'il est complexe d'accéder directement à l'effet d'une combinaison de gènes sur un caractère, il est tout à fait possible de mesurer la ressemblance phénotypique entre individus (ou covariance phénotypique) et de la relier à leur degré d'apparentement. Dès lors, il devient possible d'estimer la part génétique des facteurs influant sur le phénotype étudié. Pour se faire, on peut par exemple contrôler expérimentalement l'apparentement entre individus et extraire la part héritable d'un phénotype en utilisant une régression parent-enfant. En populations naturelles, il est possible d'estimer l'apparentement entre individus grâce à des marqueurs moléculaires co-dominants ou à l'aide d'un pédigrée (un arbre généalogique des individus de la population).

L'étude des pédigrées (résumant l'ensemble des relations d'apparentement au sein d'une population) permet de considérer un très grand nombre de paires d'individus et se fait à l'aide d'un modèle statistique à effet aléatoire (*mixed-effect models*) particulier, appelé "modèle animal". Plus précisément, le modèle animal permet de partitionner la variance phénotypique en différentes variances d'effets directement quantifiables (effets fixes: âge, sexe, climat) et en variances liée à des effets partagés entre individus (effets aléatoires: apparentement génétique). Voir l'encadré XII pour plus de précisions.

3. Que peut apporter un pédigrée aux études sur le lézard vivipare?

Dans le chapitre 2 du livre "*Quantitative genetics in the Wild* " (2014), Erik Postma s'est intéressé à l'origine des estimations d'hérabilité (voir encadre 1) que l'on peut trouver dans la littérature. Pour se faire il a effectué une vaste revue de la bibliographie entre 1970 et

Encadré XII : Principe du modèle animal

Le modèle animal propose de décomposer la variance phénotypique en plusieurs variances et d'en estimer les importances respectives. Il se base sur le modèle linéaire suivant:

$$y_i = \mu + a_i + e_i$$

où y_i représente l'écart entre le phénotype de l'individu i et le phénotype moyen de la population, μ la moyenne de la population, a_i les effets génétiques additifs (ou effets simples) pour l'individu i et e_i les effets résiduels. Pour résoudre cette équation, il faudrait connaître la valeur génétique a_i propre à chaque individu, ce qui est impossible. Par conséquent, nous ne pouvons donc pas directement estimer la part de variance expliquée par a_i dans le phénotype y_i de chaque individu.

La solution à ce problème consiste à travailler à l'échelle de la population, et de mettre en relation les variations phénotypiques entre individus et leur apparentement génétique. Pour se faire, il est possible d'utiliser un modèle linéaire particulier qui intégrera l'effet génétique comme un effet aléatoire. Ce type d'effet permet en général de gérer la non-indépendance qui peut exister entre les individus (même famille, même année de capture,...). Dans notre cas, puisque les individus sont liés génétiquement à d'autres membres de la population, il est possible de considérer leur apparentement comme source de non-indépendance et de modéliser cet effet par un effet aléatoire. Ainsi, l'effet aléatoire "valeur génétique additive" (Va) permettra de mesurer la part de variance phénotypique expliquée par l'affiliation entre individus. Si l'on passe donc à l'échelle de la population et considère l'écriture matricielle du modèle décrit plus haut on obtient :

$$y = X\beta + Zu + e$$

avec y le vecteur des écarts phénotypiques à la moyenne de la populations de chaque individu, X et Z deux matrices décrivant respectivement les effets fixes et aléatoires du modèles (0 pour l'absence d'effet ou 1 pour la présence de l'effet), β le vecteur des effets fixes, u celui des effets aléatoires, et enfin e le vecteur des effets résiduels. Parmi les effets aléatoires, on retrouvera un effet Va où A représente la matrice de relations génétiques, ou matrice d'apparentements. Pour chaque paire d'individu, il est possible de calculer un coefficient de covariance génétique dont la valeur A est de:

$$A = 2\theta_{ij}.$$

θ_{ij} représente le coefficient d'identité par descendance entre les deux individus. Il représente donc la probabilité qu'un allèle tiré aléatoirement chez l'individu i soit identique par descendance à un allèle tiré aléatoirement chez l'individu j (parents-enfants = 0.25; pleins frères = 0.125, etc.). La covariance génétique additive entre des parents et leurs descendants est donc d' $1/2 Va$. Autrement dit, chaque parent transmet la moitié de ses effets génétiques additifs à sa descendance.

Pour passer de l'échelle populationnelle à l'échelle individuelle et prédire la contribution génétique a_i au phénotype y_i de chaque individu i , il est possible de prédire l'effet moyen additif génétique à l'aide des **BLUPs** (Best Linear Unbiased Predictors) qui permettent d'obtenir une estimation des effets aléatoires pour chaque individu. Cette méthode est cependant débattue et certains auteurs recommandent de l'utiliser avec prudence (Hadfield 2010).

De nombreuses extensions de ce modèle existent, et notamment des modèles multivariés qui permettent d'étudier les effets de la sélection sur plusieurs traits corrélés. Dans ce cas, on remplace chacune des matrices par la matrice de corrélation des effets. La matrice génétique A devient matrice G , matrice de variance-covariance résumant les variances et covariances génétiques entre les individus étudiés.

2011 et s'est rendu compte que la plupart des estimations provenaient d'un nombre très limité d'espèces, elles mêmes résumées à un faible nombre de populations (488 estimations d' h^2 obtenues sur 53 espèces échantillonnées parmi seulement 76 populations, voir Figure XIII.1). Par ailleurs, sur l'ensemble de ces estimations d'héritabilité, 12 % se basaient sur l'étude d'un pédigrée. Les pédigrées chez des espèces sauvages sont donc encore rares à l'heure actuelle et sont pratiquement inexistantes chez les reptiles (ex. lézard *Uta*, *Uta stansburiana*, Sinervo et al. 2006; tortue peinte, *Chrysemys picta*, McGaugh et al. 2010; Scinque aquatique d'Australie, *Eulamprus quoyii*, Noble et al. 2014) . Pourtant, les pressions environnementales sur les ectothermes sont très fortes puisque leur physiologie dépend directement de la température du milieu. Ce taxon d'espèces semble donc un excellent modèle pour étudier les influences relatives de la génétique, de l'environnement et de leur interaction sur le phénotype.

Le site de *ROB* est suivi depuis 1989 et des prélèvements génétiques sont disponibles depuis 1993. Ainsi, il a été possible de créer un pédigrée profond et comptant plusieurs dizaines d'individus par an. Cette population a été étudiée sous de nombreux aspects (voir introduction et encadré VI), elle est donc très bien décrite et résumée par une base de données importante (9129 individus nés en élevage, 10151 individus capturés et décrits sur le terrain). Par ailleurs, elle est située dans la marge Sud de l'aire de distribution du lézard vivipare en Europe. L'augmentation des températures depuis le début du suivi est estimée à environ 3.5°C pour les mois de Mai et Juin (Chamaille-Jammes *et al.*, 2006), ayant forcément un impact sur de nombreux traits phénotypiques tels que la taille des individus (Chamaille-Jammes *et al.*, 2006), le taux de dispersion (Massot *et al.*, 2008), la date de ponte (Le Galliard *et al.*, 2010),... Par ailleurs, contrairement aux études sur les oiseaux où, en général, seul le nichoir est utilisé comme estimateur d'apparement (apparement social donc, étant donnée la proportion élevée de paternité extra-couple chez les oiseaux et en particulier chez les plus étudiés que sont les passereaux), il nous a été possible ici de créer un véritable pédigrée génétique de la population de connaître des informations à la fois sur l'effort reproducteurs des mères et des pères. De plus, puisque l'espèce est polygynandrique, une originalité de ce pédigrée est de pouvoir estimer en plus de la part des effets maternels dans la variance phénotypique transmise à la génération suivante, la part des effets paternels.

Encadré XIII : Illustrations du caractère "consanguin" des estimations d'héritabilité

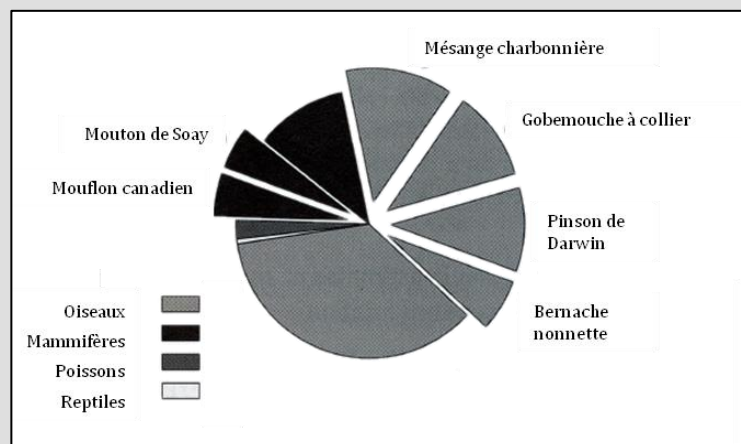


Figure XII.1. Proportion d'estimations de mesures d'héritabilité (h^2) par groupe taxonomique On remarque que la plus part des estimations proviennent de populations d'oiseaux (>75%) et que peu d'estimations ont été faites sur des ectothermes. Parmi ces estimations, la plus part sont faites sur les mêmes populations, rendant très difficile la généralisation des résultats. D'après Postma, 2014.

Pour l'ensemble de ces raisons, nous avons pensé qu'un pédigrée pourrait permettre d'aller plus loin non seulement dans l'étude de cette population, mais plus généralement dans la compréhension des mécanismes d'héritabilité et de l'importance relative de la génétique et de l'environnement. Par ailleurs, comme nous l'avons déjà mentionné, l'importance du climat sur l'ensemble de la physiologie du lézard vivipare et l'influence de la transmission d'informations maternelles durant la gestation placent la plasticité phénotypique au cœur des questionnement sur cette espèce. A terme, nous espérons, grâce à ce pédigrée tenter d'incorporer à nos modèles cette contribution conjointe de l'environnement et de la génétique.

4. Méthodes

Extraction d'ADN.

Tout individu nouvellement capturé sur le terrain ou né en élevage se voit amputé d'un petit morceau de queue (± 0.5 cm) qui est directement fixé dans une solution d'éthanol (70 %) et d'EDTA (2mM). L'extraction de l'ADN est réalisée à l'aide d'un kit de purification d'ADN QIAquick (QIAGEN®) selon le protocole fourni par la compagnie, après une digestion des échantillons de tissus, à l'aide de protéinase K à 56°C dans un tampon TE-NaCl (25mM) pendant une nuit (ou un minimum de 4 heures). Les individus sont ensuite génotypés à l'aide de 8 marqueurs microsatellites (Richard *et al.*, 2012).

Assignment de Paternité

Nous avons cherché de parfaites correspondances alléliques entre les juvéniles et une liste de pères potentiels choisis parmi une liste de mâles comprenant à la fois les mâles adultes, sub-adultes et juvéniles capturés ou nés en élevage au cours des 6 dernières années précédant la naissance du juvénile étudié. L'ensemble des assignments de paternité a été fait à l'aide du logiciel CERVUS (version 3.0). De plus, lorsque le (ou les) père(s) n'éta(ien)t pas connu, nous avons cherché à isoler les pleins frères des demi-frères en cas de paternité multiple. La correspondance avec les mères n'a été faite que par simple vérification puisque chaque femelle est isolée dans l'élevage, et par conséquent forcément la mère des petits retrouvés dans son terrarium (à quelques rares exceptions près). Pour l'instant, nous n'avons pas cherché à trouver les parents de nouveaux individus capturés sur le terrain. Voir Richard *et al.* 2012 pour plus de précisions dans la méthode d'assignment.

5. Description du pédigrée

Le pédigrée dans sa version actuelle comprend 5373 individus capturés ou nés en élevage entre 1993 et 2015 (parents). Plus exactement, il contient 4532 juvéniles nés à partir de 1999, issus de 943 portées et de 696 mères connues (certaines femelles sont présentes jusqu'à 4 fois en élevage). L'analyse de leurs génotypes nous a permis de trouver 379 pères (certains identifiés plusieurs années et accouplés avec plusieurs femelles). Nous connaissons au total 1389 familles dont 792 avec deux partenaires identifiés (57%). Parmi les adultes, 187 mères et 129 pères sont nés dans notre élevage (soit 27% et 34 % des adultes du pédigrée, respectivement).

CHAPITRE 5: Variation de l'estimation de l'héritabilité génétique additive en fonction de la qualité du pédigrée.

Pour m'approprier le pédigrée et ses caractéristiques et pour en décrire les caractéristiques, j'ai voulu réaliser un premier papier théorique portant sur les variations dans les estimations des mesures d'héritabilité en fonction du nombre de père renseigné dans le pédigrée. J'ai donc simulé une série de pédigrée, dérivé du pédigrée réel et calculé plusieurs paramètres d'héritabilité à l'aide de plusieurs décomposition de variance phénotypique. D'après la littérature, je m'attends à ce que l'augmentation des mauvaises assignations diminue les mesures d'héritabilité (Charmantier and Réale, 2005).

Les résultats sont cependant surprenant puisqu'ils vont à l'encontre des résultats classiquement trouvés. Des vérifications et des analyses complémentaires sont en cours pour valider ces conclusions.

*Article 4: Influence of polyandry on heritability estimates made from a wild *Lacertidea* pedigree*

Work in progress

Authors : Alexis Rutschmann¹, Murielle Richard¹, Anne Charmantier² & Jean Clobert¹

¹ CNRS, USR2936, Station d'Ecologie Théorique et Expérimentale, 2 route du CNRS, F-09200 Moulis, France;

² CNRS, Centre d'Ecologie Fonctionnelle et Evolutive, 119 route de Mende, F-34293 Montpellier cedex 5, France.

Abstract :

Quantitative genetic studies play an important role in the understanding of the evolutionary processes in natural populations. However, these studies are mainly based on wild avian populations where extra-pair paternity (EPP) often exists. Mainly social pedigrees are used to describe relatedness among individuals which often ignore multi-paternity events and lead to erroneous estimations of heritabilities (h^2). Previous work has suggested that EPP should cause minor under-estimation of h^2 if the pedigree is deep and if extra-pair copulations are random and not influenced by the trait under focus. However, if EPP is relatively low in avian species (5% to 40%) multi-paternity can also reflect a reproductive strategy for some species where female multiply reproductive partners. In this paper, we explored the impact of multi-paternity on h^2 estimations in the common lizard (*Zootoca vivipara*), a species where multipaternity is the rule rather than the exception.. To do so, we compared h^2 from genetic pedigree and from several simulated pedigree with progressive levels of paternity misassignment.

Keywords : Polyandry, misassigned paternity, pedigree error quantitative genetic, *Zootoca vivipara*.

INTRODUCTION

L'application récente des modèles de génétique quantitative aux populations naturelles a permis d'appréhender d'un œil neuf l'évolution de la variation génétique au cours du temps et d'estimer son importance face à la sélection naturelle (Lynch & Walsh, 1998). Parmi les différentes approches permettant d'estimer l'importance de cette variabilité génétique dans la variance phénotypique (*i.e.* héritabilité), l'utilisation du modèle animal est de plus en plus privilégiée puisqu'elle permet de tenir compte des apparentements entre de grands nombres d'individus en résumant leur proximité génétique par une matrice génétique, elle-même issue d'un pédigrée de la population (Kruuk, 2004).

La plupart des estimations d'héritabilité en populations naturelles retrouvées à l'heure actuelle dans la littérature proviennent de populations d'oiseaux (revue dans Merilä and Sheldon 2001). Si ce groupe présente de nombreux avantages pratiques pour la mise en place de suivis de populations à long terme (identification aisée des parents sociaux, accès aux juvéniles dès la naissance,...) plusieurs sources de biais liés à la biologie de ces espèces sont susceptibles d'affecter les estimations d'héritabilité. Par exemple, le parasitisme de nid peut créer des erreurs dans l'assignement des parents à un juvénile, et ainsi augmenter de façon artificielle la variation phénotypique présente au sein d'un groupe considéré comme apparenté. Ce cas de figure reste cependant relativement rare (Griffith *et al.*, 2002) et n'affecte les estimations d'héritabilité que dans une moindre mesure. Des erreurs plus importantes peuvent provenir de la paternité extra-couple (EPC, *extra pair copulation*) qui imposera des cas de multi-paternité indécélables en étudiant la population uniquement selon sa structure sociale. La fréquence d'EPC varie entre espèce mais est reconnue comme importante chez la plupart des passereaux étudiés à l'aide de modèles animaux (Moller & Ninni, 1998; Griffith *et al.*, 2002) mais pourrait concerner jusqu'à 55% des petits d'une femelle chez certaines espèces (Dixon *et al.*, 1994). L'EPP a deux effets principaux sur les estimations d'héritabilité. D'abord, si elle n'est pas renseignée, elle fausse les relations père-enfants d'une portée et modifie ainsi le degré d'apparentement entre petits d'une même portée. Autrement dit, elle regroupe artificiellement sous un même degré d'apparentement génétique des variations phénotypiques en fait indépendantes. D'autre part, un choix non

aléatoire de pères extra-couples par les femelles (ex. mâle de meilleure qualité) peut mener à une sous-estimation de la covariance génétique entre un père et ses petits. Quoi qu'il en soit, il en résulte une sous-estimation de l'héritabilité puisqu'une part de la variation phénotypique de la population sera malencontreusement attribuée aux effets génétiques d'un père unique plutôt qu'à plusieurs.

Plusieurs études se sont déjà penchées sur ce problème, en mêlant très souvent une approche empirique et de la modélisation (Milner *et al.*, 2000; Charmantier & Reale, 2005; Bérénois *et al.*, 2014; Firth *et al.*, 2015). La plupart d'entre elles s'accordent à dire que des sous-estimations importantes de l'héritabilité apparaissent en cas d'importantes EPP. Ces dernières seront d'autant plus importantes si les traits étudiés sont directement influencés par le degré d'EPP de la ponte. En effet, qu'il s'agisse d'EPP ou non, certains traits vont être directement influencés par le nombre de pères participant à la reproduction d'une seule femelle.

Un autre scénario, moins étudié, pourrait lui aussi engendrer des erreurs similaires. Si le phénotype de la portée est "modifié" par la mère à l'image de celui d'un des pères de la portée (*female imprinting*, FI), on s'attend à une mauvaise estimation des mesures d'héritabilité. En effet, dans un tel cas on tiendrait compte de l'ensemble des pères participants à la reproduction au lieu de n'utiliser que le père contribuant réellement au phénotype des petits. Autrement si, si tous les pères sont considérés, des liens génétiques existant, mais ne participant pas à l'élaboration du phénotype des petits, vont venir expliquer la variance phénotypique observée au sein de la portée. Cette part de variance génétique apportée par les pères sera non-informative pour le modèle et diminuera donc l'héritabilité. Au contraire, si l'on considère uniquement le père qui sert de modèle génétique à l'ensemble de la portée, la part de variance phénotypique expliquée par la génétique sera cette fois-ci bien prise en compte et sa part expliquant augmentera.

Au cours de cette étude nous avons voulu utiliser le pédigrée assemblé au cours de ma thèse pour traiter de la question sous un angle nouveau. En effet, le lézard vivipare (*Zootoca vivipara*) présente de fort taux de multi-paternité. Si certains individus se contentent d'un seul partenaire sexuel par reproduction, la polygynandrie semble représenter une véritable stratégie chez d'autres qui cumulent jusqu'à 3 partenaires reproducteurs (Richard *et al.*,

2005). Dans ce contexte il est indispensable d'utiliser une approche d'identification génétique des individus lors de la création d'un pédigrée puisqu'elle permet de tenir compte de la multi-paternité des juvéniles.

Plus précisément, nous avons voulu estimer l'impact que pouvait avoir une mauvaise assignation de paternité sur les estimations d'héritabilité de la taille à la naissance des juvéniles. Pour se faire, nous avons utilisé plusieurs pédigrées dérivés du pédigrée réel de la population que nous échantillons génétiquement depuis 1999:

P1/ Pédigrée réel

P2/ Pédigrée où le mâle le plus commun dans la ponte est père de l'ensemble de la ponte.

P3/ Pédigrée 2, en conservant les paternités non assignées comme inconnue.

P4/ Pédigrée 2, en conservant le second mâle le plus commun comme père de ses petits.

P5/ Pédigrée 4, en conservant les paternités non assignées comme inconnue.

Cette approche nous permet non seulement de comprendre à quel point l'utilisation de pédigrées sociaux peut perturber les estimations d'héritabilité mais aussi de comprendre l'influence de la multi-paternité sur ces derniers.

MATERIEL ET METHODES

Echantillonnages et création du pédigrée

Les lézards sont issus d'une population située sur le Mont Lozère dans le Massif Central (44°30'N 3°45'S, 1420m altitude) que nous suivons depuis 1988. Après capture, les femelles sont amenées à l'élevage où elles sont isolées jusqu'à parturition dans des conditions standards (Rutschmann et al. 2015). Cet élevage individuel des femelles permet d'établir directement le lien entre une mère et ses petits. A leur naissance, les juvéniles sont mesurés (jSVL) et sexés.

Tout individu nouvellement capturé sur le terrain ou né en élevage est génotypé (± 0.5 cm de tissu prélevé à l'extrémité de la queue fixé dans une solution d'éthanol à 70% et d'EDTA

2mM). L'extraction de l'ADN est réalisée à l'aide d'un kit de purification d'ADN QIAquick (QIAGEN®) selon le protocole fourni par la compagnie, après une digestion des échantillons de tissus, à l'aide de protéinase K à 56°C dans un tampon TE-NaCl (25mM) pendant une nuit. Les individus sont ensuite identifiés à l'aide de 8 marqueurs microsatellites (Richard et al. 2012).

Pour les assignations de paternités, nous avons cherché de parfaites correspondances (aucun mismatch) entre les juvéniles et une liste de pères potentiels choisis parmi une liste de mâles comprenant à la fois les mâles adultes, sub-adultes et juvéniles capturés ou nés en élevage au cours des six dernières années précédant la naissance du juvénile étudié. Six ans nous semble une bonne limite puisqu'il est rare que les mâles adultes dépassent cet âge. L'ensemble des assignations de paternité a été fait à l'aide du logiciel CERVUS (version 3.0). De plus, lorsque le (ou les) père(s) n'étai(en)t pas connu, nous avons cherché à isoler les pleins frères des demi-frères en cas de paternité multiple (Logiciel Kingroup v2, Konovalov et al. 2004). Voir Richard et al. 2012 pour plus de précisions dans la méthode d'assignation.

Le pédigrée complet comprend 5373 individus capturés ou nés en élevage entre 1993 et 2015 (parents). Plus exactement, il contient 4532 juvéniles nés à partir de 1999, issus de 943 portées et de 696 mères connues (certaines femelles sont présentes jusqu'à 4 fois en élevage). L'analyse de leurs génotypes nous a permis de trouver 379 pères (certains identifiés plusieurs années et accouplés avec plusieurs femelles). Nous connaissons au total 1389 familles dont 792 avec deux partenaires identifiés (57%). Parmi les adultes, 187 mères et 129 pères sont nés dans notre élevage (soit 27% et 34 % des adultes du pédigrée, respectivement).

Création des pédigrées alternatifs

Nous avons créé plusieurs pédigrées alternatifs reflétant chacun différents niveaux de fiabilité des paternités.

P1: Le pédigrée sans aucune modification. Les pères non identifiés sont entrés comme valeurs manquantes dans le pédigrée.

P2: Pour chaque portée, seul le père majoritairement représenté est conservé. Il remplace l'ensemble des autres pères, connus ou inconnus. En cas d'égalité, l'un des deux pères est tiré au sort comme père principal.

P3: Identique à P2 mais les pères non identifiés restent non identifiés.

P4: Pour chaque portée, les 2 pères les plus représentés sont conservés. Le père majoritaire remplace les autres pères et les pères non déterminés. En cas d'égalité, les pères conservés sont tirés au hasard.

P5: Identique à P4 mais les pères non identifiés restent non identifiés.

Estimation d'héritabilité

Nous avons calculé les héritabilités de la taille des juvéniles à la naissance ($jSVL$), trait pour lequel nous possédons le plus d'informations. Nous avons préféré la taille juvénile à la taille adulte pour deux raisons principales : d'abord, c'est la classe d'âge pour laquelle nous avons le plus d'individus. Ensuite, la croissance étant continue chez les Lacertidea (Shine, 2005), nous voulions minimiser l'influence de la variance environnementale sur nos estimations d'héritabilité. Par ailleurs, si les individus ne sont pas nés en élevage, ou capturés à l'âge sub-adulte, il est complexe de déterminer leur âge avec précision et donc de comparer justement leur taille.

Les estimations de la variance génétique additive (V_a), de la variance environnementale (V_E), de la variance des effets maternels (V_M) et de la variance résiduelle (V_R) composant la variance phénotypique (V_P) pour la taille à la naissance ont été faites à partir d'une analyse par inférence bayésienne des paramètres d'un modèle animal (Kruuk 2004). A partir de ces paramètres, il est possible d'estimer l'héritabilité au sens stricte du terme tel que: $h^2 = V_a/V_P$ avec $V_P = V_a + V_M + V_R$. Nous avons utilisé le package MCMCglmm (Hadfield) et une méthode de Monte-Carlo par chaînes de Markov (MCMC) pour estimer les variances d'intérêts. Pour limiter l'auto-corrélation, nous avons choisi de faire tourner les chaînes 125.000 fois après un préchauffage de 25.000 itérations. Les enregistrements sont effectués toutes les 10 itérations. De cette façon, l'auto-corrélation entre les itérations ne dépasse jamais 0.015. La distribution à priori de la V_a renseignée dans ce modèle bayésien, est une distribution non informative Wishart inverse ($\alpha \cdot \mu = 0$, $\alpha \cdot V = 0$), renseignée par défaut, et dans laquelle nous plaçons peu de confiance ($\nu = 0.002$). Pour chaque simulation, nous avons calculé les DIC afin de trouver le meilleur modèle.

Quatre modèles sont testés sur chaque pédigrée. Le modèle 1 décompose la variance dans la taille à la naissance entre une composante additive génétique et une composante résiduelle.

Le modèle 2 décompose la variance dans la taille à la naissance entre une composante additive génétique, un effet maternel et une composante résiduelle. Le modèle 3 décompose la variance dans la taille à la naissance entre une composante additive génétique, une composante liée à l'année de naissance et une composante résiduelle. Le modèle 4 comprend l'ensemble des effets.

RESULTATS

Les estimations d'héritabilités et de la variance génétique additive pour la taille des individus à la naissance sont synthétisées dans la table 1. Les résultats indiquent des différences entre les mesures d'héritabilité calculées à l'aide des pédigrées simulés et les héritabilités calculée à partir du pédigrée génétique. Ces différences sont peu marquées pour les modèles 1 et 3. Au contraire elles sont importantes pour les modèles 2 et 4 qui tiennent compte des effets maternels.

En fonction des pédigrées, le modèle retenu à l'aide du DIC varie lui aussi. Pour 4 des 5 pédigrées simulés, le modèle expliquant le mieux la variance phénotypique comprend les effets maternels.

Table 1: Estimation d'héritabilité et de variance génétique additive pour chaque modèle et chaque pédigrée. Sont renseignés les estimations moyennes de chaque paramètre et les écarts-types entre parenthèses. Nous donnons aussi un intervalle de confiance à 95% pour les estimations de h^2 . Enfin, nous présentons le DIC de chaque modèle afin d'évaluer, pour chaque pédigrée, le modèle donnant la meilleure estimation des paramètres d'héritabilité.

	Modèle 1				Modèle 2				Modèle 3				Modèle 4			
	h^2	95% CI	Va	DIC	h^2	95% CI	Va	DIC	h^2	95% CI	Va	DIC	h^2	95% CI	Va	DIC
P1	0.68	0.64/0.74	3.72	17352	0.38	0.31/0.47	2.38	17310	0.33	0.22/0.44	2.97	17307	0.16	0.09/0.24	1.53	17540
P2	0.70 (0.02)	0.64/0.74	3.8 (0.18)	17145	0.65 (0.03)	0.59/0.70	3.84 (0.2)	16842	0.34 (0.06)	0.21/0.45	2.97 (0.18)	17117	0.34 (0.05)	0.23/0.44	3.07 (0.19)	16953
P3	0.68 (0.02)	0.64/0.73	4.01 (0.2)	17360	0.65 (0.03)	0.58/0.71	3.98 (0.21)	17302	0.33 (0.05)	0.22/0.45	2.97 (0.18)	17307	0.33 (0.06)	0.22/0.45	2.89 (0.18)	17534
P4	0.69 (0.02)	0.64/0.74	3.8 (0.18)	17340	0.64 (0.03)	0.58/0.70	3.8 (0.21)	17173	0.34 (0.05)	0.22/0.46	3.11 (0.2)	17307	0.32 (0.05)	0.22/0.44	3.08 (0.21)	17212
P5	0.69 (0.02)	0.64/0.74	3.7 (0.18)	17234	0.65 (0.03)	0.59/0.70	3.78 (0.19)	16935	0.34 (0.06)	0.23/0.46	3.04 (0.18)	17188	0.33 (0.06)	0.22/0.44	3.01 (0.19)	17002

DISCUSSION

Estimation d'héritabilité

Les estimations d'héritabilité (h^2) varient entre les différents modèles. Pour le pédigrée génétique, elles diminuent de moitié environ pour chaque élément de variance ajouté au modèle (0.68, 0.38, 0.33 et 0.16 dans l'ordre des modèles). Cet effet est lié à une meilleure décomposition de la variance phénotypique et à l'absorption d'une part de la variance génétique additive par les variances maternelles ou annuelles. Ce résultat nous renseigne d'abord sur la présence d'effets maternels forts qui conditionnent le phénotype des petits. Ces effets sont bien connus chez le lézard vivipare et il n'est pas surprenant qu'ils soient reflétés dans nos modèles (Meylan and Clobert 2004; Bleu et al. 2013, Bestion, *in prep*). Ensuite, des différences environnementales entre années ainsi que des différences entre les femelles dans leur capacité à gérer ces variations existent et influent de façon importante sur la qualité des juvéniles (Rutschmann et al., *in prep*). Encore une fois, il est donc normal de les retrouver à ce niveau.

Perspective 1 : il serait intéressant de comparer l'apport des effets environnementaux et maternels obtenus dans cette étude avec ceux obtenus dans les études utilisant d'autres pédigrées génétiques (passereaux : Charmantier and Reale 2005; cerfs : Kruuk et al. 2002; moutons de Soay : Milner et al. 2000). Ceci nous permettrait de comparer la part de variance phénotypique expliquée par l'environnement chez un ectotherme et chez des endothermes.

Perspective 2 : les pères se reproduisent le plus souvent avec plusieurs femelles et un certain nombre a pu être identifié, il serait donc intéressant d'ajouter un effet paternel et de voir comment il influe sur l'héritabilité. En effet, de nombreux petits répartis entre différentes portées et parfois sur différentes années possèdent un même père qui pourrait, à l'instar des femelles, homogénéiser leur phénotype. A notre connaissance, détecter un tel effet serait une nouveauté dans une étude sur une population animale sauvage.

Héritabilité et multi-paternité

Le résultat principal que l'on peut tirer de cette étude est que la multi-paternité force, dans certains cas, une surestimation des coefficients d'héritabilité. Ces résultats sont en totale contradiction avec les études similaires que l'on peut trouver dans la littérature (Charmantier & Reale, 2005; Firth *et al.*, 2015). En effet, l'ensemble de ces études montrent que lorsque les pères sont mal renseignés, les taux d'héritabilité sont sous-estimés (jusqu'à 15% environ, Charmantier & Reale, 2005). Les proportions dans lesquelles les héritabilités sont ici surestimées varient cependant entre les modèles. Si les différences sont négligeables pour deux d'entre eux (entre 1 et 5 % pour le modèle 1, entre 0 et 1 % pour le modèle 3), elles atteignent des proportions importantes pour les modèles 2 et 4 (25% et 14 % respectivement). Ces modèles comprennent tous les deux un effet maternel dans leur structure. Ainsi, lorsque l'on extrait la variance liée à l'effet maternel, une mauvaise assignation du père a des conséquences importantes.

A l'heure actuelle, nous ne pouvons pas dire si les résultats observés sont le fruit d'un effet biologique ou d'une erreur liée à la formalisation des modèles animaux ou des pédigrées. Nous espérons pouvoir rapidement lever le doute sur cette question avec l'aide d'Anne Charmantier. En attendant, il est tout de même possible d'avancer une explication biologique à ces résultats, notamment dans un cas où l'un des deux parents manipule la portée de façon à l'homogénéiser. Par exemple, si la femelle calque l'ensemble des phénotypes sur le phénotype des petits du mâle dominant, nous nous trouvons dans un contexte où la variance phénotypique et la variance génétique apportées au modèle par les autres mâles reproducteurs ne serait alors que du bruit statistique affaiblissant la véritable relation parents-enfants. Il en résulterait une diminution de l'héritabilité mesurée. Au contraire, lorsque seul le mâle majoritaire est considéré, le bruit phénotypique disparaît et l'héritabilité augmente. Les différences plus fortes trouvées en tenant compte d'un effet maternel renforcent ce questionnement sur une éventuelle manipulation du phénotype des jeunes par la femelle.

Perspective 3 : Pour vérifier si cet effet n'est pas artéfactuel il est possible de comparer simplement les variances phénotypiques entre pontes et d'observer la réaction de cette variance face à la multi-paternité. Si notre hypothèse est exacte, nous nous attendons à ce que la variance reste constante, quel que soit le nombre de pères.

Héritabilité et degré de mal-assignation

Contrairement aux résultats trouvés par Charmantier et al., (2005), le degré de mauvaise assignation ne semble pas influencer particulièrement les estimations d'héritabilité. En effet, qu'un, deux ou l'ensemble des pères soient présents, ou que les jeunes de pères inconnus soient attribués à un père ou restent sans paternité, n'affecte pas significativement nos estimations. Au vu du nombre de familles décrites dans le pédigrée (943 familles, 4532 juvéniles), et vu l'importance que semblent avoir les effets maternels, il est probable qu'une mauvaise assignation des pères soit anecdotique dans le calcul des héritabilités.

Conclusions

Ces analyses ne sont encore que préliminaires et malheureusement nous ne pouvons préjuger de la robustesse des résultats obtenus. Nous ne ferons donc pas de conclusions hâtives et espérons pouvoir présenter un travail plus abouti au moment de la soutenance. Quoi qu'il en soit, le pédigrée semble fonctionnel et les estimations d'héritabilité obtenues nous donnent de bons espoirs quant à sa puissance et aux nombreuses opportunités qu'il nous offre.

DISCUSSION GENERALE

Dès mon master je me suis particulièrement intéressé au rôle que pourrait jouer la plasticité phénotypique dans le maintien des espèces face aux changements globaux. J'ai d'abord vu ce mécanisme comme un nouvel espoir face à la menace que représente l'augmentation des températures liée au changement climatique. En effet, la plasticité semble faciliter les invasions biologiques (Davidson *et al.*, 2011), procurer des réponses rapide à une modification du milieu (Ghalambor *et al.*, 2007b), élargir la niche des espèces (Duputié *et al.*, 2015) et pour certains auteurs être une condition indispensable à l'adaptation des espèces à de nouveaux milieux (Aubret, 2015). Au fur et à mesure de mes recherches et de mes lectures, je me suis rendu compte qu'il y avait de nombreuses limites à son développement ou son efficacité (Murren *et al.*, 2015). Lorsqu'il a fallu redéfinir en partie le sujet de ma thèse face aux difficultés que nous rencontrions pour monter le pedigree, c'est tout naturellement que je me suis replongé dans ces questions. Le jeu de données monté par l'équipe depuis 30 ans possédait en effet plusieurs avantages qui me permettaient d'étudier la plasticité phénotypique en population naturelle. D'abord sa double échelle spatiale et temporelle qui permet grâce aux différentes populations réparties dans le Massif Central de comprendre le rôle des conditions environnementales sur l'évolution des normes de réactions et, grâce au suivi à long terme, de se concentrer sur les mécanismes à l'origine de ces différences. Indéniablement, un autre avantage vient du modèle d'étude, le lézard vivipare, dont pratiquement l'intégralité de son fonctionnement est sous l'influence du climat.

La plasticité phénotypique a connu un fort regain d'intérêt au cours de la dernière décennie grâce au rôle qu'elle pourrait jouer face au changement climatique. Les études qui se concentrent sur elle (et dont nous avons largement discuté jusqu'à présent) cherchent essentiellement à comprendre quel est (et sera) l'impact des conditions environnementales sur l'évolution de la plasticité et quelles sont (et seront) les limites de son évolution. Si les prédictions théoriques sont de plus en plus précises et intègrent de plus en plus de scénarii, les études qui les confirment en populations naturelles sont encore rares. En profitant du jeu

de données mis à ma disposition, j'ai pu (en partie du moins) apporter quelques preuves empiriques à ces modèles.

Réponse des populations aux variations de l'environnement

Dans le premier chapitre, j'ai pu démontrer que les normes de réactions des populations réparties dans le Massif Central variaient en fonction des combinaisons de facteurs locaux. Dans un premier temps, la température moyenne, qui influence directement les performances métaboliques des individus des différentes populations, semble forcer la différenciation des réponses phénotypiques moyennes que l'on observe sur chacun des sites. Dans un second temps, la variance autour de cette moyenne va elle aussi jouer un rôle crucial. Selon les prédictions théoriques, je m'attendais à ce que la réponse plastique augmente avec la variance environnementale mais les résultats indiquent le contraire. Rappelons que la variance mesurée ici est une variance thermique journalière et il est très compliqué, voire impossible, de prédire cette variation à l'avance, particulièrement en milieu montagneux. Néanmoins, si les variations de températures sont faibles à l'échelle historique, les individus peuvent profiter d'une réponse plastique qui sera fiable dans la majorité des situations. Au contraire, si les variations environnementales sont trop importantes, et surtout peu prévisibles, le phénotype produit par la réponse plastique aura plus de chance d'être loin du phénotype optimal et donc d'être mal-adapté. Dans ce genre de situation, une réponse plus robuste aux variations environnementales (réponse fixe) sera avantagée. Ces résultats confirment donc en partie les hypothèses selon lesquelles variabilité environnementale et prédictibilité du milieu sont indispensables à l'évolution d'une réponse plastique adaptative. Ils illustrent également l'importance de considérer l'impact de l'homme sur son milieu : les pontes sont plus tardives dans les zones couvertes par la végétation et plus précoces en zones ouvertes par l'anthropisation. Cette observation est certainement due à un effet rafraichissant de la couverture végétale qui disparaît avec l'ouverture du milieu. De plus, un effet d'interaction démontre que l'effet de la température est accentué dans les milieux ouverts. Ces résultats démontrent donc que l'évolution des normes de réactions est sous l'influence de facteurs multiples de l'environnement et qu'il est maladroit de se baser sur la réponse d'une seule population pour tirer des conclusions sur la réponse d'une espèce au changement climatique.

Malheureusement ces travaux ne suffisent pas à démontrer une adaptation locale des individus à leur milieu et il faudrait effectuer par exemple des expériences de transplantations réciproques d'individus pour confirmer cette théorie. Il serait possible en effet qu'une acclimatation des individus aux conditions locales permette des réponses différentes entre populations. Les expériences que j'ai pu mener en laboratoire vont dans ce sens. J'ai en effet tenté de mettre en évidence des différences entre populations pour plusieurs caractéristiques physiologiques individuelles telles que les températures préférentielles des individus ou leur vitesse d'augmentation (ou de diminution) de la température corporelle. Ces expériences nécessitent d'être répliquées mais, pour le moment, aucun signal de différenciation n'apparaît entre populations. Plusieurs facteurs peuvent expliquer ces résultats. D'abord une importante hétérogénéité des poids des individus, qui masquerait le signal entre populations au profit d'un signal fort entre les individus. Ce problème peut cependant être réglé statistiquement sans trop de difficulté. Ensuite, le fait que les femelles soit gestantes. Ce stade de vie est connu pour contraindre particulièrement l'état physiologique et hormonal des femelles, ce qui pourrait homogénéiser les réponses entre populations (Gvozdík & Castilla, 2001; Meylan & Clobert, 2004). Enfin, et je vois ici l'explication la plus probable, le fait que les individus passent un temps important dans l'élevage dans des conditions relativement chaudes par rapport au milieu naturel pourrait homogénéiser l'ensemble des réponses (Pintor, 2015). Cet effet n'est pas rare et a fait l'objet de plusieurs travaux. On estime par exemple que 2 semaines suffisent aux serpents pour acclimater leur taux métabolique à de nouvelles conditions (Blem & Blem, 1990). Même si ces vitesses ne sont pas uniformes entre les espèces (de 0 à 7 semaines, référence dans Pintor 2015), le rôle que pourrait jouer l'acclimatation dans notre cas est potentiellement important. Il reste compliqué de dire si cette acclimatation des populations sera suffisante pour palier à une augmentation des températures dans les décennies à venir et si elle suffira à effacer l'influence du climat sur l'ensemble de la physiologie du lézard vivipare. En effet, les différences en termes de gestations sont marquées et des signaux significatifs de différenciations entre les normes de réactions des différentes populations ont pu être détectés malgré un passage de plusieurs semaines dans l'élevage. Des expériences complémentaires, menée cette fois ci directement à l'arrivée en élevage ou de transplantation réciproque (exemple dans Lorenzon et al. 2001) pourrait nous permettre de répondre en partie à ces interrogations.

Le second chapitre confirme les résultats obtenus dans le premier. Bien que l'effet anthropique n'ait pas joué un rôle ici et a donc été écarté des analyses, les variations thermiques ont encore une fois un effet sur la relation étudiée. Plus les conditions sont chaudes, plus les contraintes liées à l'investissement énergétique dans la reproduction semblent s'effacer et moins la taille de la portée n'impacte sa qualité. Cet effet est retrouvé à deux échelles spatiales. Au sein des populations d'abord, avec un effet atténuateur des températures sur l'intensité du compromis. Entre populations ensuite, puisque les populations les plus froides de notre suivi sont contraintes quelle que soit la gamme d'environnements qu'elles rencontrent alors que les populations les plus chaudes peuvent profiter de l'effet positif de la température sur le trade-off. Ces résultats démontrent dans un premier temps que l'observation d'une corrélation nulle (voire positive) entre deux traits supposés interagir négativement l'un sur l'autre n'est pas le signe de l'absence de trade-off. La variation de la robustesse du compromis illustre simplement que des contraintes (ex. environnementales, physiologiques, génétiques) influent aussi sur l'expression de chacun des traits impliqués dans le compromis. Ce type d'effet est par exemple retrouvé dans un phénomène appelé "*silver spoon effect*" qui décrit l'avantage reproducteur ou en terme de survie dont profitera un individu qui bénéficie de ressources abondantes à un stade précoce de sa vie (Van De Pol *et al.*, 2006). Par ailleurs, cette analyse confirme qu'il est primordial d'étudier la réponse des ectothermes sur l'ensemble de la gamme thermique que rencontre l'espèce avant de tirer des conclusions générales. En se concentrant uniquement sur la norme de réaction d'une population, les prédictions faites par les modèles cherchant à prédire le sort des espèces face aux défis climatiques qui les attendent dans les décennies à venir risquent d'être erronées. Au contraire, s'il est possible d'implémenter l'étendue des variations des normes de réactions dans ces modèles mécanistiques, leurs prédictions seront plus fiables.

Réponses individuelles aux variations de l'environnement

Le chapitre 3 se consacre essentiellement aux réponses individuelles et à leur variation à l'intérieur de la population ROB. Si l'observation de différences interindividuelles des normes de réaction est devenue classique dans la littérature (Brommer *et al.*, 2005), il est encore rare de trouver des études qui s'intéressent aux origines de ces variations et à leur caractère adaptatif dans la population. Dans le cas du lézard vivipare, nous avons pu

détecter un lien entre les variations de normes de réactions et le climat : plus les individus sont dans des conditions chaudes, plus les réponses sont intenses et plus les écarts entre les normes de réactions des individus sont importants. En conditions froides au contraire les individus présentent des normes de réaction plus similaires, certainement à cause des conditions plus restrictives imposées par des températures basses. Pour aller plus loin nous avons cherché à traduire ces différences de normes de réactions en avantage adaptatif. A l'échelle de la population, l'avancement des dates de ponte permet une croissance plus importante des juvéniles avant leur hibernation et par conséquent une meilleure survie (Bestion et al. 2015). A l'échelle individuelle cependant, une réponse trop plastique semble être désavantageuse. Les résultats indiquent en effet que les femelles les plus plastiques grandissent moins entre deux saisons de reproductions et donnent naissance à des petits en moyenne moins gros. Il y aurait donc un avantage à être plastique, mais jusqu'à un certain point seulement. Au delà de celui-ci, la plasticité entre en conflit avec d'autres traits d'histoire de vie. Nous n'avons pas pu explorer l'impact direct de la plasticité sur la survie des femelles car les effectifs étaient trop faibles pour mener correctement une analyse CMR. Sur les juvéniles cependant, les quelques modèles préliminaires que j'ai pu effectuer semblent démontrer que la plasticité des femelles n'influence pas la survie des juvéniles jusqu'à l'âge sub-adulte. Des études complémentaires sur ces sujets pourraient cependant être menées sur les individus présents dans le Métatron de la station. Ce dispositif expérimental abrite à l'heure actuelle plusieurs populations de lézards vivipares réparties dans des cages de 100m², selon différents scénarios de réchauffement climatiques. En utilisant les données des femelles et de CMR beaucoup plus précises (3 événements de captures par été, très faible taux de fuite d'individus,...) il serait possible d'estimer le coût de la plasticité sur la survie et la reproduction des femelles et des juvéniles, le tout en mesurant l'effet de températures extrêmes.

Il serait également intéressant d'utiliser le pédigrée que nous avons pu mettre en place pour estimer le degré de corrélations génétiques entre les différents traits étudiés (plasticité, taille à la naissance, etc). Couplées avec les caractéristiques thermiques rencontrées par chaque individu, ces analyses pourraient nous renseigner sur les conditions dans lesquels ces corrélations sont exprimées et nous permettre d'estimer à quel point elles peuvent contraindre l'évolution des populations dans le contexte de changement climatique. Par

ailleurs, elles pourraient nous renseigner sur le degré d'héritabilité de la réponse plastique entre générations.

Augmentation des températures et conséquences sur la dynamique des populations

Même si les effets de l'augmentation des températures que nous observons semblent à priori bénéfiques (avancement des dates de pontes, levée des contraintes sur l'investissement reproducteur, ...), plusieurs indices suggèrent qu'ils pourraient devenir délétères si le réchauffement des populations persiste. Des résultats expérimentaux obtenus dans le Métatron par notre équipe ont pu démontrer qu'une augmentation des températures accélérerait le cycle de vie des lézards et augmenterait le risque d'extinction des populations (Bestion *et al.*, 2015). Si l'on s'intéresse plus précisément aux mécanismes sous jacent, on se rend compte que dans les populations de cette expérience, les juvéniles sont plus lourds à la naissance, ont des performances thermiques plus élevées et un rendement métabolique plus important. Ces changements de phénotypes influencent les stratégies d'histoire de vie adultes et entraînent par exemple une reproduction plus précoce (reproduction de sub-adultes, plusieurs reproduction par saison) et moins contrainte. Paradoxalement, les individus vont se dépenser plus dans un contexte où les opportunités d'acquérir des ressources diminuent (Sinervo *et al.*, 2010). Au final, il en résulte une diminution de la survie à l'âge adulte et un basculement de la dynamique de population vers des cycles plus rapides qui à terme augmentent le risque d'extinction des populations.

Les analyses que j'ai menées sur des données collectées en populations naturelles tendent à confirmer ce travail expérimental. La relaxe du trade-off entre qualité et quantité de juvénile semble par exemple bénéfique pour la reproduction en cours mais risque d'accentuer le trade-off entre la reproduction présente et la reproduction future (chapitre 2). Il serait intéressant de confirmer cette tendance grâce à un suivi des reproductions des femelles dans le temps. Ce travail serait notamment possible sur la population *ROB* et je m'attends à ce que des épisodes de relaxe du trade-off (s'ils existent sur cette population) perturbent les futurs épisodes de reproduction et la survie des adultes dans les années qui suivent.

Ensuite, comme nous l'avons fait remarquer dans le chapitre 3, les variations interindividuelles dans les normes de réaction pour la date de parturition sont plus importantes les années chaudes. Dans ce type d'année, la période d'activité journalière se

retrouve réduite puisque les températures en milieu de journées dépassent plus fréquemment les limites supportables par les individus que dans des années plus fraîches. Si certains individus peuvent éviter ces températures en s'abritant dans des zones plus favorables de leur habitat, cette option n'est pas forcément disponible pour tous (Kearney *et al.*, 2009). Il y a lors plusieurs cas possible. Dans le meilleur des cas, les individus trouvent dans leur territoire une zone où la température reste proche de la température optimale de performance (zone humide ou ombragée) et continue leur activité sans souffrir de la chaleur. Si une telle zone n'est pas disponible les individus risquent de souffrir d'une accélération métabolique liée à l'augmentation des températures, consommant beaucoup d'énergie malgré une acquisition des ressources ne qui suit pas. La réponse plastique est alors non adaptative et reflète une contrainte purement métabolique qui entraîne, comme le suggère nos résultats, une réduction de la taille des juvéniles à la naissance et une limité dans l'investissement de la femelle dans sa propre croissance.

Pour mieux mesurer l'impact des températures chaudes sur l'activité des lézards, nous avons initié avec l'aide de M. Vickers et de D. Miles un suivi thermique précis de chaque lézard sur l'ensemble des sites de capture. Un dispositif d'enregistrement thermique déployé pour la première fois à l'été 2015 nous permet désormais de mesurer la température tous les mètres, toutes les minutes sur un transect de 25m de longueur (75m dès 2016). Grâce à ce dispositif nous pouvons caractériser tout au long de la journée les variations thermiques auxquelles font face les lézards sur une vaste gamme d'habitat (herbe sèche, herbe humide, sous couvert végétale, en milieu ouvert, rocher, buisson,...). De plus, à chaque capture, nous enregistrons l'heure, la température du lézard, la température et le type d'habitat qu'il utilisait avant de fuir. De cette façon nous avons pu remarquer par exemple que la température au sol pouvait dépasser 48°C sur certains substrat aux heures les plus chaudes de la journée, période où seuls quelques individus en mauvais état (masse faible, charge parasitaire importante) s'aventuraient hors de leur trou. Cette température dépasse de plus de 6° la température maximale supportée par le lézard vivipare (42°C; Van Damme *et al.*, 1991) et de presque 15°C la température optimale pour plusieurs activités (voir Introduction). A l'aide de ces données nous sommes en mesure de simuler des cartes thermiques représentant l'habitat potentiel que rencontrent les lézards sur chaque population et d'étudier la façon dont les individus utilisent cet habitat. Nous espérons

Encadré XIV: Température et croissance individuelle:

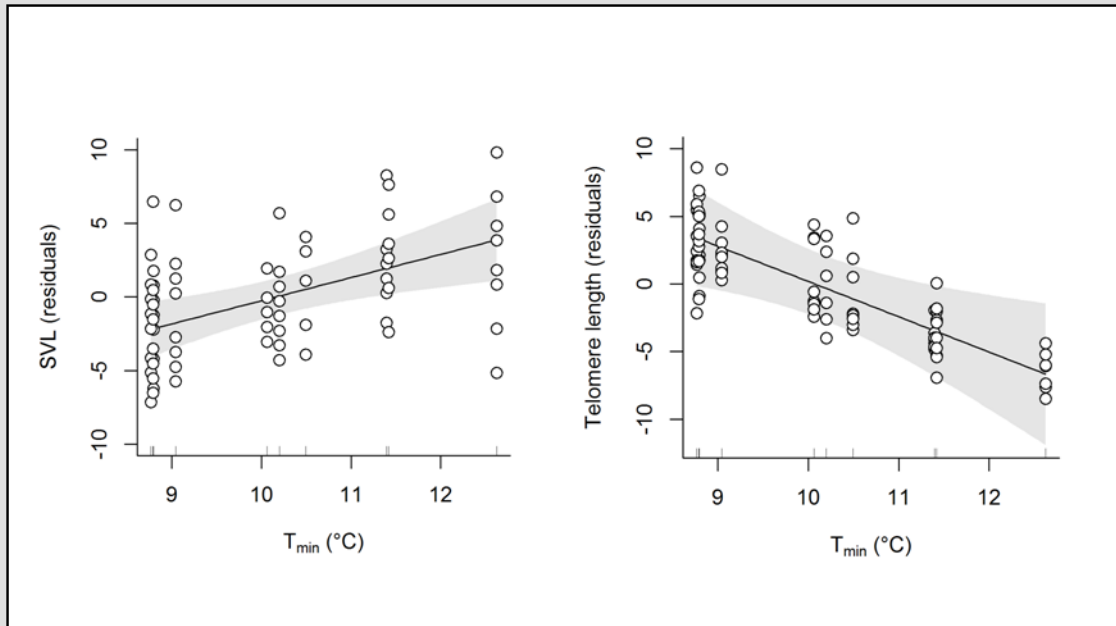


Figure XIII.1: Influence des températures minimales de l'été sur la taille des sub adultes et la longueur de leurs télomères.

notamment pouvoir mieux appréhender l'effet des variations de températures régissant chaque population et comprendre comment les individus utilisent leur territoire thermique pour en maximiser leurs performances physiologiques au cours de la journée. Cette approche est originale puisque plutôt que de se placer dans un paradigme où seules les températures froides limitent l'activité des lézards ("cost-benefit model", Huey and Slatkin 1976), elle considère aussi les températures chaudes comme une limite potentielle (Vickers 2011). Avec ce changement de paradigme, de nombreux travaux prédisent une extinction des espèces d'ectothermes face au réchauffement à venir (Sinervo et al., 2010 mais voir Clusella-Trullas et al. 2011). Ils se basent en général sur la forme des courbes de

performances thermiques des espèces étudiées pour prédire le risque d'extinction des espèces (voir Figure VI.2). Malheureusement, la plupart des études qui se focalisent sur ces questions utilisent un nombre très restreint d'individus pour formuler leur conclusion (pour leur défense, l'acquisition de telles données nécessitent des protocoles très lourds). Encore une fois, un biais statistique évident apparaît ici et rend dangereux la généralisation des résultats. Tout au long de ma thèse, nous avons continué un projet initié par Don Miles qui visait à calculer des courbes de performances de course de tous les mâles capturés sur les populations du suivi Global Change. En couplant ces données avec (1) les cartes thermiques mise en place avec M. Vickers, (2) avec des modèles simulant l'utilisation de cet espace qu'il développe à l'heure actuelle et (3) avec des données individuelles sur l'efficacité des lézards à augmenter ou diminuer leur température corporelle (voir table X.1), nous espérons pouvoir mieux appréhender les effets des températures critiques (hautes ou basses) sur les populations de lézards vivipares. A terme, nous aimerions déterminer si la variance interindividuelle qui existe à l'heure actuelle dans les différents environnements que nous échantillons suffira pour que les populations répondent positivement à la sélection imposée par l'augmentation des températures à venir.

D'autres études sont en cours pour mieux appréhender ces effets des hautes températures. Avec A. Dupoué, S. Meylan et J.F. Le Gaillard nous avons initié un projet visant à mesurer directement le taux de stress métabolique des individus. Nous avons prélevé des échantillons sanguins dans 8 populations au cours de l'été 2015 et avons par exemple pu estimer la longueur des télomères des cellules plasmatiques. Plus ces télomères sont courts, plus la division cellulaire est intense et plus le taux métabolique est élevé. Les premiers

résultats indiquent un effet net des températures minimales sur leur longueur, ce qui confirme nos hypothèses. En restant hautes tout au long de la journée ou de la nuit, elles forcent une activité physiologique continue pour les individus, ce qui augmente leur état de stress (voir figure XIV.1). Nous avons par la même occasion initié un suivi des sub-adultes plus poussé afin d'estimer la croissance de cette classe d'âge au cours de l'été et de la comparer entre populations. Ces données ont commencé à être collectées en 2015 qui, par "chance" se trouve être l'une des années les plus chaudes que la région ait pu connaître (avec 2003). Nous espérons qu'à terme l'équipe pourra ainsi estimer les effets de tels épisodes de chaleur sur la dynamique des populations du suivi.

Autres effets environnementaux

Comme nous l'avons vu dans le chapitre 1, d'autres facteurs peuvent jouer un rôle crucial sur les réponses du lézard vivipare. Par exemple, l'anthropisation qui accentue les effets des températures sur la période de gestation. Dans le Massif Central, de plus en plus de milieux sont ouverts suite à une reprise de l'activité pastorale et si les températures augmentent avec le changement global, nous pouvons craindre que l'effet de cette augmentation soit décuplé par l'ouverture des milieux. Comme nous venons de le signaler, l'anthropisation sur nos populations est essentiellement liée à une pression de pâturage (ovins, caprins, équins). Un impact indirect de ces animaux est leur tendance à être vecteur de parasites, qu'ils peuvent transmettre dans leur environnement. Nous avons étudié cette question avec Q. Wu et nous sommes rendus compte qu'effectivement la charge parasitaire des individus était influencée par la présence d'animaux d'élevage dans les populations. Même si les effets sur les individus ne sont pas clairs, les parasites semblent tout de même affecter la reproduction (voir chapitre A2 en annexe). Enfin, avec A. Dupoué, nous avons estimé l'impact du degré d'humidité environnementale sur certaines populations de notre suivi. Nous avons pu détecter des différences entre individus sur la façon de gérer ce stress (i.e. évapotranspiration, osmolalité) et de potentiels effets de stress hydrique plus marqué dans les populations chaudes. Ces analyses sont toujours en cours mais suggèrent que l'ensemble des caractéristiques du milieu influencent la valeur sélective des individus. Même s'il est plus aisé des les étudier l'un après l'autre pour mieux en comprendre les effets, une intégration aussi large que possible des effets environnementaux est nécessaire pour estimer à quel point les populations sont susceptibles de souffrir des changements globaux en cours.

Encadré XIV. Augmentation des températures : impact sur le lézard vivipare.

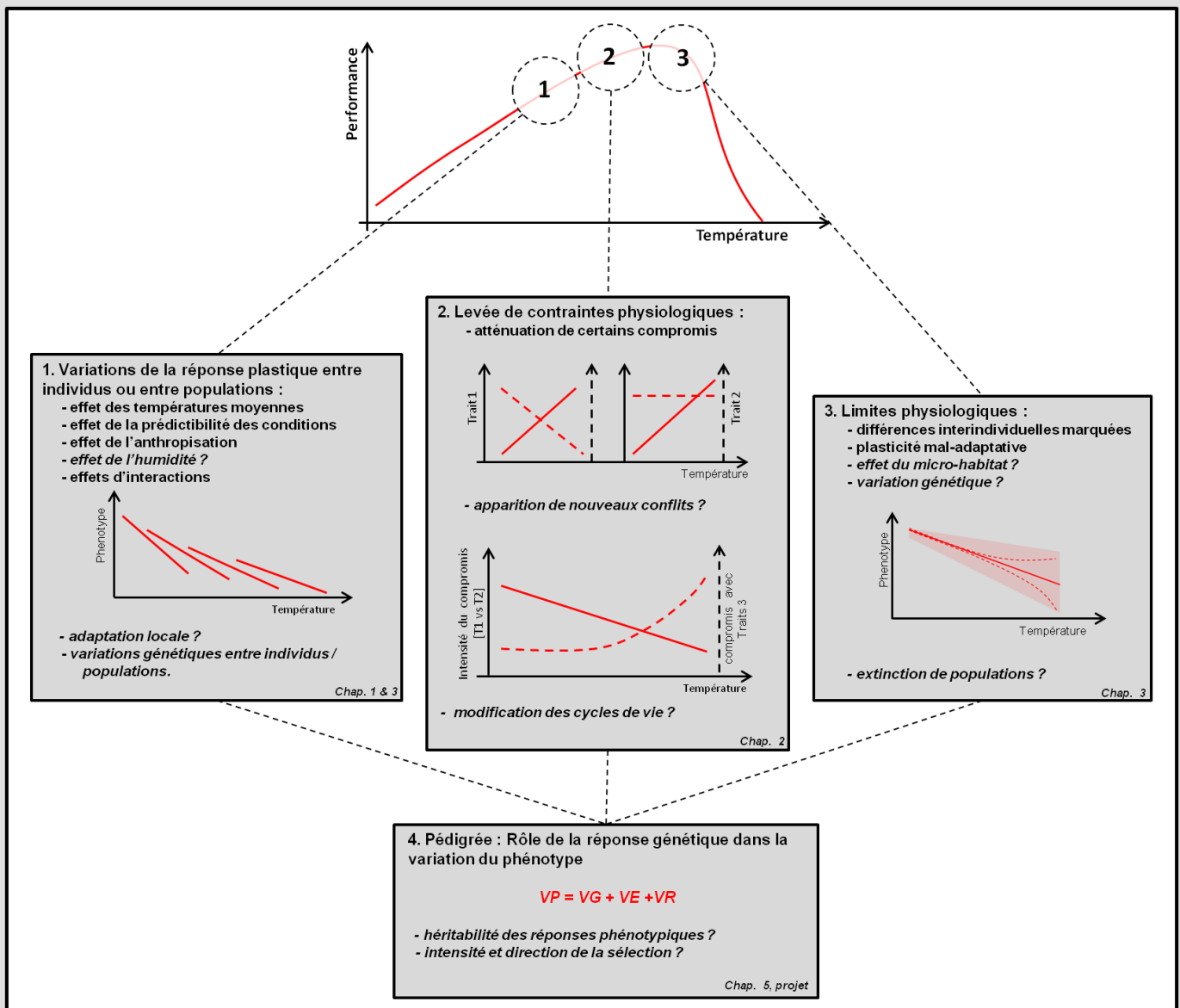


Figure XIII.1: L'augmentation des températures va améliorer progressivement les performances des individus jusqu'à un optimal thermique au delà duquel elles auront un effet délétère. (1) Les individus et les populations présentent des différences dans leur relation plastique avec les températures. En effet, de nombreuses variables environnementales telles que les températures moyennes, la variabilité des températures, l'anthropisation (et leurs interactions) vont jouer sur l'intensité de cette réponse. (2) Si les températures s'approchent suffisamment de l'optimum thermique, les performances vont être meilleures et certaines contraintes peuvent être levées. Cette effet bénéfique pour certains phénotypes vont cependant pouvoir affecter d'autres paramètres individuels, et créer de nouveaux conflits affectant à leur tour les stratégies d'histoire de vie des individus. (3) Lorsque les températures deviennent trop chaudes, les phénotypes produits peuvent être mal-adaptatif et les performances individuelles en être réduites. Des différences interindividuelles, liées aux caractéristiques physiologiques des individus ou à leur territoire vont accentuer ou diminuer les effets de telles températures. (4). L'utilisation d'un pédigrée nous permettrait de mieux comprendre comment et pourquoi la sélection fluctue en fonction des conditions du milieu et quel rôle joue l'héritabilité génétique des phénotypes dans la variance observée au sein des populations naturelles.

SYNTHESE ET CONCLUSION

Il serait possible de synthétiser mon travail de thèse par le schéma XIV. Comme nous l'avons vu, un ectotherme dépend pour chaque phénotype de la température du milieu. La qualité du phénotype peut être résumée par une courbe de performance en cloche asymétrique (Fig. IV.1). Lorsque la température augmente, les performances augmentent progressivement jusqu'à s'effondrer rapidement si la température optimale est dépassée. Au cours de la thèse j'ai pu démontrer différents effets de la température le long de cette courbe de performance. D'abord, j'ai pu démontrer que les variations de multiples facteurs locaux (température, prédictibilité du climat, anthropisation, humidité,...) ainsi que leur interactions peuvent influencer sur la forme de la norme de réaction des individus et des populations (chapitre 1 et 3, Fig. XIV.1). J'ai aussi pu démontrer que lorsque les températures s'approchent de l'optimum thermique, certaines contraintes peuvent être levées. Je suspecte cependant que cet effet, par un mécanisme de levier, entraîne l'apparition de nouveaux compromis pouvant altérer le cycle de vie des individus (chapitre 2, Fig. XIV.2). Enfin, lorsque les températures sont trop élevées, les individus vont souffrir d'une diminution de leurs performances et les réponses risquent d'être mal-adaptatives. La façon dont chaque individu est affecté par les températures extrêmes semble encore une fois varier et pourraient notamment dépendre de la qualité de leur territoire (chapitre 3, Fig. XIV.3). Enfin, et même si cet aspect est encore à l'état de projet, j'ai pu terminer l'assemblage d'un pédigrée recouvrant plus d'une dizaine de génération. J'aimerais l'utiliser pour comprendre comment l'intensité de la sélection varie en fonction des conditions climatiques mais aussi pour estimer le rôle de l'héritabilité des normes de réaction dans l'évolution des populations de lézard vivipare (chapitre 5, Fig XIV.4).

Pour conclure, je pense avoir montré au cours de cette thèse que différents traits d'histoire de vie du lézard vivipare varient en fonction des caractéristiques de son environnement et ce à plusieurs échelles d'intégration. L'ensemble des variations semblent connectées et les mécanismes influençant les variations entre les populations se retrouvent entre individus. Pour aller plus loin, j'espère pouvoir profiter du pédigrée mis en place pour étudier l'impact des fluctuations environnementales au niveau génétique.

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ANNEXES

Les travaux présentés en annexe sont le fruit de collaborations auxquelles j'ai participé au cours de ma thèse. Mon apport sur ces analyses a été à la fois dans la collecte des données, l'analyse statistique, l'analyse des résultats et l'écriture ou la relecture des manuscrits. Suivent les deux manuscrit parus au cours de ma thèse dans leur version publiée.

CHAPITRE A1: Contraintes environnementales et stress hydrique.

Ce chapitre expérimental est né d'une collaboration avec Andréaz Dupoué, Jean-François Le Galliard et Sandrine Meylan. En essayant de caractériser les populations du suivi et de comprendre l'impact du changement climatique sur le lézard vivipare, il est apparu clair que le facteur humidité, largement sous estimé dans nos études pouvait avoir un rôle clef. Chez les reptiles, l'humidité relative du milieu est un facteur qui peut influencer de façon importante la physiologie des animaux et qui peut notamment être très restrictif au cours de la gestation. L'étude comparative de populations d'une même espèce peut permettre d'estimer à quel point ce facteur est restrictif: il suffit en effet de mesurer à quel point les individus sont adaptés à un gradient de disponibilité du dit facteur pour évaluer son importance.

En profitant du suivi que je menais sur Global Change, nous avons voulu vérifier si les lézards présentaient des syndromes d'adaptations au niveau de disponibilité hydrique, et notamment confirmer la relation négative attendue entre la perte hydrique et l'aridité du milieu. Pour cela, nous avons expérimentalement calculé un taux de perte en eau pour chaque lézard capturé au cours de l'année 2015. Nous l'avons ensuite comparé aux caractéristiques de l'environnement de capture de chaque lézard. Par ailleurs, au sein de chaque population, nous avons voulu vérifier si l'effort reproducteur des femelles influençait ces pertes hydriques.

Face à plusieurs défauts méthodologiques de notre approche et à la vu d'autres résultats, nous avons cependant décidé de laisser ce papier de côté pour le moment. En effet, une analyse de l'osmolalité plasmatique (proportion d'eau dans le sang) est en cours et nous renseignera sur l'état hydrique des individus. D'autre part, une analyse de la taille des télomères des individus sur chacune des populations est en passe de se terminer. Elle nous permettra de caractériser les environnements les plus stressants pour le lézard vivipare.

La version de l'article que nous avons prévu de publier est tout de même présentée ici.

Article 4 : Environmental gradients and reproductive effort correlate with water losses in natural populations of the Common lizard (*Zootoca vivipara*).

For consideration in: Comparative Biochemistry and Physiology A, in progress

Authors : Andréaz Dupoué , Alexis Rutschmann, Donald Miles, Jean François Le Galliard, Jean Clobert, Sandrine Meylan

Abstract

Functional responses to energy accessibility in the environment have been extensively studied in past decades, and in comparison the adaptations or the acclimations to water availability are still relatively overlooked. Water is a crucial resource that directly impacts species survival and reproduction, and the access to this resource is expected to change in relation to climate change (e.g., summer droughts, dryspell, etc). Although some water-conserving strategies have been documented, few studies investigated the relationships between water balance and microclimatic conditions in the same temperate species. In this study, we examined the variation of water balance (i.e., plasma osmolality and water loss) in males and females common lizards (*Zootoca vivipara*) from 13 natural populations during summer, along natural gradients in temperature and humidity. We found that males were more dehydrated than females, a result probably linked to a difference in thermoregulatory and activity behaviors over gestation since the rate of water loss was similar between sexes. Then, we found that minimal conditions better correlated to physiological traits and to reproductive performance than mean or maximal conditions. Regarding the effects of those conditions, we found interactive effects of temperature and humidity on osmolality in males and on reproductive effort in females. The opposite relationships of osmolality and water loss, and reproductive effort against humidity between cool and warm populations suggest that the pattern we found might reflect the balance between microclimatic effects and physiological adaptations/acclimation (i.e., impermeability). We emphasized that further integrative studies should improve our understanding in factors influencing the water balance from physiological to behavioral adaptations and/or acclimation. These steps are notably essential to improve the accuracy of models predicting species responses to climate change.

Keywords. Altitude, humidity, lizard, reproductive effort, temperature, water loss

Summer variation of water balance along microclimatic gradients in natural populations of the Common lizard (*Zootoca vivipara*)

Andréaz Dupoué ^{1*}, Alexis Rutschmann ², Donald Miles ³, Jean François Le Galliard ^{1,4}, Jean Clobert ², Dale F. DeNardo ⁶, Sandrine Meylan ^{1,5}

¹ CNRS/UPMC, UMR 7618, iEES Paris, Université Pierre et Marie Curie, 7 Quai St. Bernard, 75005, Paris, France

² Station d'Ecologie Expérimentale du CNRS à Moulis, USR 2936, 09200 Saint Giron, France

³ Department of Biological Sciences, Ohio University, Athens, Ohio, 45701, USA

⁴ CNRS/ENS UMS 3194, CEREEP – Ecotron IleDeFrance, École Normale Supérieure, 78 rue du Château, 77140 St-Pierre-lès-Nemours

⁵ ESPE de Paris, Université Sorbonne Paris IV, 10 rue Molitor, 75016 Paris, France

⁶ School of Life Sciences, Arizona State University, Tempe, AZ 85287-4501, USA

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Keywords. Dehydration, ectotherm, humidity, osmolality, pregnancy, temperature, water loss.

INTRODUCTION

Water is a vital resource that may influence many aspects of species functional traits from physiological performance such as locomotion, growth, immunity, or reproduction (Wilson & Havel, 1989; Lorenzon *et al.*, 1999b; Marquis *et al.*, 2008b; Moeller *et al.*, 2013), to survival (Whitehead *et al.*, 1996; Taylor *et al.*, 2006; Rittenhouse *et al.*, 2009; Zylstra *et al.*, 2013). Surprisingly, despite its ecological relevance, water has received relatively little attention in ecological studies compared to other resources such as temperature, energy or time (Bonnet & Brischoux, 2008). As demonstrated elsewhere, the maintenance of water balance (i.e., the balance between hydration state and water losses) might help in understanding and predicting general pattern such as distribution (Dunkin *et al.*, 2013; Peterman & Semlitsch, 2014), demography (Foley *et al.*, 2008; Marquis *et al.*, 2008b; McKechnie & Wolf, 2010; Dey *et al.*, 2015) and / or habitat use (Davis & Denardo, 2009; Guillon *et al.*, 2014). Species water balance may be challenged as soon as water availability is restricted, and therefore the clarification of environmental or physiological factors affecting the water balance may be particularly relevant regarding the changes in climatic conditions (e.g., increase or decrease in the rate of precipitations and / or the increase in drought and dryspell frequency or intensity; Easterling *et al.* 2000; IPCC 2015). This is especially true in terrestrial ectotherms due their high physiological sensitivity to external conditions and given their strong vulnerability to climate change (Deutsch *et al.*, 2008; Dillon *et al.*, 2010).

A diversity of studies have previously focused on the water saving strategies including changes in activity (Lorenzon *et al.*, 1999b; Davis & Denardo, 2009; Hetem *et al.*, 2010), shifts in thermoregulatory strategies (Crowley, 1987; Lorenzon *et al.*, 1999b; Ladyman *et al.*, 2006; Angilletta *et al.*, 2010; Köhler *et al.*, 2011), and/or metabolic depression (Kennett & Christian, 1994; Tieleman *et al.*, 2002; Muir *et al.*, 2007). These adjustments aim at lowering the rate of evaporative water loss (Moen *et al.*, 2005) and eventually the rate of dehydration. Still, few studies have investigated variation of hydration state in the wild and accent has mainly been restricted on factors influencing the rate of water loss. For instance, a well described pattern so far is the negative relationship between the rate of water loss and the habitat aridity (Tieleman *et al.*, 2003; Williams *et al.*, 2004; Moen *et al.*, 2005; Van Sant *et al.*, 2012; Johnson *et al.*, 2013; Guillon *et al.*, 2014; Cox & Cox, 2015). That is, species from arid environments are less permeable than species inhabiting mesic or humid environments.

Temperature may also influence the rate of water loss either through lower skin resistance or higher metabolic rate (Webster *et al.*, 1985; Lillywhite, 2006; Dupoué *et al.*, 2015a) and yet, comparative studies of water losses along thermal gradient are rare. Besides, some physiological states can also impacted the rate of water loss and hydration state (Cohen, 1975; Dupoué *et al.*, 2015a). Eventually, water balance of breeding individuals may be particularly challenged. For instance, females are often exposed to multiple constraints over pregnancy/gravidity including modified behavior (e.g., increase thermal preference; Lorient *et al.*, 2013; Shine, 2006), physiology (e.g., higher metabolic rate; Dupoué and Lourdais, 2014; Schultz *et al.*, 2008), and morphology (e.g., higher physical burden; Le Galliard *et al.*, 2003; Shine, 1988) that can induce in return higher rate of water loss through ventilation and transpiration (Webster *et al.*, 1985; Woods & Smith, 2010; Dupoué *et al.*, 2015a). Furthermore, all over their development, embryos may require consequent amount of water due to somatic growth (Du, 2004; Lourdais *et al.*, 2015) and eventually, those requirements may lead to mother-offspring conflict whenever access to water is compromised (Dupoué *et al.*, 2015b). However, the integrative of temperature, humidity and reproductive performance on the water balance are poorly documented in wild individuals.

In this study, we examined the variations of hydration state and water loss in a viviparous lizard species (*Zootoca vivipara*) using males and pregnant females from 13 natural populations distributed along a thermal and humidity gradients in the Massif Central region (France). This ectothermic species is particularly appropriate to investigate the effects of environment on water balance due its high sensitivity to water availability. Water has previously been shown to influence several physiological and behavioral adjustments in this species. For instance, a previous descriptive study in the same area has shown that the level of precipitations may have immediate effect on offspring survival, and delayed effect on female reproductive performance (Marquis *et al.*, 2008b). Similarly, experimental restriction of water in this species during pregnancy may dramatically impaired reproductive success (Dauphin-Villemant & Xavier, 1986) while slighter restriction may interact with post-natal environments to impacts individuals (Lorenzon *et al.*, 2001).

Herein, we focused on two functional traits related to water balance: plasma osmolality (i.e., the indicator of hydration state in species with no salt-gland, Peterson, 2002) and the rate

water loss, and we tested their correlation with microclimatic conditions and reproductive performance. We hypothesize the hydration state to reflect adaptation or acclimations to microclimatic conditions as well as physiological states (i.e., pregnancy). We first expect females to be higher dehydrated than males due to the hydric constraints over pregnancy described above; that is a higher rate of water loss and the intergenerational tradeoff for water (Dupoué *et al.*, 2015a, 2015b; Webber *et al.*, 2015). Then, we predict the plasma osmolality to be negatively correlated with habitat humidity while the correlation with the rate of water loss should be positive. That is individuals from dry populations should be more dehydrated (high osmolality value) than individuals from humid populations and they should be less permeable (lower rate of water loss) to limit the dehydration rate. Conversely, we predict the plasma osmolality to be positively correlated with habitat temperature while the correlation with the rate of water loss should be negative. That is individuals from cool populations should be less dehydrated (low osmolality value) than individuals from warm populations and they should be more permeable (higher rate of water loss) due to lower level of constraints. In females, we also predict positive correlation between the rate of water loss and the reproductive effort.

MATERIAL AND METHODS

Studied species, populations description and housing

The common lizard, *Zootoca vivipara*, is a small widespread Lacertidae (adult snout-vent length ~ 50 - 75 mm) inhabiting humid habitats across northern Eurasia. This viviparous species lives in peat bogs and heath lands. In the studied populations, males emerge in mid-April while females emerge in early May. Emergence is followed by mating, fertilization, and immediately by ovulation and pregnancy. Pregnancy lasts 2 to 3 months and parturition occurs from mid-July to early August. Litter size usually vary from 1 to 12 newborns and after birth, neonates do not received any post-natal parental care. After parturition, females are strongly emaciated and restore energy reserves before entering into hibernation in late September.

We caught 246 females (mean $\pm$ SE, body mass (BM) = 4.84 ± 0.07 g, snout-vent length (SVL) = 61.44 ± 0.24 mm) and 135 males (BM = 3.47 ± 0.06 g, SVL = 54.30 ± 0.31 mm) from 13 populations between the 19th and the 26th of June 2015 (Table 1). All studied

populations have been sampled in the Massif Central mountain region (France), at the southern limit of the species' range (Pilorge *et al.*, 1983). These populations are distributed along different levels of elevation which result in different thermal and humidity conditions (Fig. 1, Table 1). In each population, we recorded temperature and humidity using 2 to 3 data loggers (Thermochron iButtons[®], and Hygrochron iButtons[®], with a resolution of 0.0625°C and 0.04% Relative Humidity - RH) placed on the ground into micro-habitats in which we found lizards in high density. Because RH varies with temperature, we used the water density (in g.m⁻³) as an index of absolute humidity with the approximation of a stable barometric pressure of 1013.25 mbar (see details in Tieleman *et al.*, 2002). The temperature and humidity were recorded every hour and we standardized the sampling period in analyses from 29th June to 17th July to compare the populations. Over this sampling period, we extracted the daily mean, minimal and maximal temperature (respectively T_{mean} , T_{min} , and T_{max}) and humidity (H_{mean} , H_{min} , and H_{max}) to assess the microclimatic conditions of each population (Table 1). Based on the population T_{min} , we distinguished two thermal profiles: cool populations ($T_{\text{min}} < 9.5^{\circ}\text{C}$) and warm populations ($T_{\text{min}} > 9.5^{\circ}\text{C}$).

Few hours after capture, males and females were transferred to a field laboratory. Due to limited place in the field laboratory, we could not house all individuals and thereby males from 5 populations and females from one population were not brought back in laboratory (Table 1). All other individuals were kept in an individual terrarium (11 x 18 x 11 cm) with a sterilized soil layer and a shelter. Heat and water were provided following a standard protocol (Massot & Clobert, 2000b). Lizards were allowed to thermoregulate under a bulb (25W) for 6 h a day (from 09.00 to 12.00 h and from 14.00 to 17.00 h). We provided water 3 times a day and we fed lizards with 2 crickets every two days. In females, we recorded the litter mass (i.e., the mass of all neonates) just after parturition because water loss are likely to correlate with reproductive effort. Within three days after parturition, we released females with their litter at the exact capture location. Due to other experiments, we released males at the end of July at the exact capture location.

Plasma osmolality

Within the 5 minutes after capture, all lizards were bled using a well-established protocol (Meylan *et al.* 2003). Blood samples (40-60µl whole blood) were collected from the post-orbital sinus using 2-3 20µl microcapillaries. In the lab, blood samples were centrifuged for

5min (3000 rpm), plasma was separated from blood cells and kept frozen in airtight tubes until analyses. Plasma osmolality was then determined using a vapor pressure osmometer (model 5500, Wescor, Logan, UT, USA). Before analyses, plasma was diluted (1:1) in a Ringer solution (300 mOsm.kg⁻¹) previously prepared (see detailed composition in Secor et al., (1994), so that plasma osmolality was determined from 10µl duplicates (intra-individual variation: 3.9%). Procedure of osmolality measure followed then the protocol described in Wright et al., (2013).

Water loss estimations

The day of capture, all lizards were weighed (BM₁) and then maintained under stable thermal (23.5 ± 0.06°C) and humidity (14.6 ± 0.04 g.m⁻³) conditions, without any access to water or food. After 24 hours, all individuals were weighed again (BM₂) and we estimated the rate of water loss (in mg.h⁻¹) using the loss of mass (BM₂ – BM₁) during this period. We used body mass loss as a proxy of total evaporative water loss (i.e., the sum of respiratory and cutaneous evaporative water losses) because in squamate reptiles, mass variations are highly representative of variation in water loss (DeNardo *et al.*, 2004; Moen *et al.*, 2005; Lillywhite *et al.*, 2008; Johnson *et al.*, 2013; Dupoué *et al.*, 2015b). However, we did not control for feces mass for technical reasons related to husbandry priorities. Still, regarding the previously reported mass of feces in this species (mean, males: 37.9 mg, females: 52.2 mg; González-Suárez et al., 2011) and the mass change we attributed to the loss of water in this study (mean ± SE, males: 168.2 ± 13.2 mg, females: 153.8 ± 5.7 mg), the feces mass would have represented significant proportion of the mass loss (males: 22.5%, females: 33.9%) and therefore would have been detected. For instance, after previous investigations on the dataset, we excluded 2 females from analyses because they showed extremely high values of mass loss (655 and 1059 mg) possibly due to feces or egg loss.

Statistical analyses

All analyses were performed with R software (R Development Core Team, version 3.2.0, <http://cran.r-project.org/>). First, we used linear models to test the effects of SVL, populations and sex and their interaction on the plasma osmolality. Second, we investigated the effect of microclimatic conditions within each sex on the plasma osmolality. We performed the analyses within each sex because we did not sample males and females from all populations

(Table 1) and because males and females differ in their physiological states (respectively non-breeding vs. pregnant). We used mixed-effects linear models (package nlme) in which population identity was set as random factor to account for repeated measurements within the same population. Temperature (T_{mean} , T_{min} and T_{max}), humidity (H_{mean} , H_{min} and H_{max}) and reproductive performance were treated as linear covariates (Table 2), thereby those covariates were previously centered. We then used the Akaike's information criterion (AIC) to compare the models including the covariates alone compared to their additive or their interactive effects (Table 2). Two models were considered different when their AIC differed from 2 ($\Delta\text{AIC} > 2$; Burnham and Anderson, 2002) and when $\Delta\text{AIC} < 2$, we retained the most parsimonious models (with the fewer parameters). When we found a significant interaction between temperature and humidity, we used models in which temperature profile (i.e., warm *versus* cool populations) was treated as fixed factor and humidity as linear covariate to facilitate results interpretation and visualization.

We used the same approach to analyze the variation in the rate of water loss, and in female reproductive performance (Table 2 & 3). We considered 3 indexes of reproductive performance: the embryonic development (ED; estimated as the number of days between sampling date and parturition date), the absolute reproductive effort (ARE; estimated as the mass of all neonates) and the relative reproductive effort (RRE, derived from the linear relationship between litter mass and female size, $F_{1,169} = 89.5$, $p < 0.001$, $r^2 = 0.35$).

Finally, we used mixed-effects linear models with population as a random factor to test the correlation between the plasma osmolality and the rate of water loss. In females, we used this approach to test the correlation between plasma osmolality or water loss and reproductive performance.

RESULTS

1) Plasma osmolality

Population and sex effects

Plasma osmolality was marginally impacted by sex (mean $\pm$ SE, males: 311.4 ± 3.3 mOsm.kg⁻¹, females: 302.0 ± 2.6 mOsm.kg⁻¹, $F_{1,242} = 3.37$, $p = 0.068$) and influenced by population

($F_{9,242} = 2.22$, $p = 0.022$), and by the interaction between population and sex ($F_{9,242} = 2.75$, $p = 0.004$), and it was not influenced by the lizard SVL ($F_{1,242} = 1.42$, $p = 0.234$).

Correlations with microclimatic and reproductive covariates

In both sexes the retained models included the effects of minimal thermal and humidity conditions (Table 2). In males, we found no influence of $T_{\min}$ ($F_{1,6} = 1.11$, $p = 0.333$) and $H_{\min}$ ($F_{1,6} = 0.12$, $p = 0.736$) but a significant influence of the interaction term ($\beta = 9.02 \pm 3.28$, $F_{1,6} = 7.58$, $p = 0.033$). In cold populations, the plasma osmolality was negatively correlated with the $H_{\min}$ ($\beta = -13.49 \pm 5.45$, $t_{1,6} = -2.48$, $p = 0.048$, Fig. 2), while it was positively correlated in warm ones ($t_{1,6} = 14.78$, $t_{1,6} = 3.14$, $p = 0.020$, Fig. 2). In females, although models including the effects of minimal and mean microclimatic conditions or the effects of embryonic development and reproductive effort were better than the null model (Table 2), we did not find any influence of $T_{\min}$, $H_{\min}$, their interaction, neither of the ED, or the estimates of reproductive effort (ARE and RRE) on the plasma osmolality (all $p > 0.234$).

Interaction between thermal profile and the rate of water loss

In males, plasma osmolality was influenced by population thermal profile (cool: 315.2 ± 6.0 mOsm.kg⁻¹, warm: 316.7 ± 6.1 mOsm.kg⁻¹, $F_{1,5} = 6.80$, $p = 0.048$), and by the rate of water loss ($F_{1,52} = 4.29$, $p = 0.043$), and the interaction term ($F_{1,52} = 9.56$, $p = 0.003$). In cool populations, the plasma osmolality was positively correlated with the rate of water loss ($\beta = -3.06 \pm 1.47$, $t_{1,52} = -2.07$, $p = 0.043$, Fig. 3), while we found an opposite pattern in warm populations ($\beta = 3.36 \pm 1.46$, $t_{1,52} = 2.30$, $p = 0.025$, Fig. 3). In females, we did not find any effect of thermal profile, water loss or their interaction on the plasma osmolality (all $p > 0.302$, Fig. 3).

2) Rate of water loss

Population and sex effects

The rate of water loss was significantly impacted by the population ($F_{5,202} = 6.08$, $p < 0.001$), and the interaction between population and sex ($F_{5,202} = 3.15$, $p = 0.009$), but it was not

influenced by the sex (mean $\pm$ SE, males: 6.87 ± 0.54 mg.h⁻¹, females: 6.52 ± 0.23 mg.h⁻¹, $F_{1,202} = 0.15$, $p = 0.700$) or the lizard SVL ($F_{1,202} = 1.71$, $p = 0.193$).

Correlations with microclimatic and reproductive covariates

In males, the model comparisons retained the solely influence of $T_{\min}$ ($\beta = 1.58 \pm 0.55$, $F_{1,4} = 8.25$, $p = 0.045$, Fig. 4) while in females, the null model was not different from the others (Table 2). That is, in females, the rate of water loss did not correlate with temperature, humidity, their interaction, neither with the reproductive performance (all $p > 0.142$). However, when investigating the effect of $T_{\min}$ on the rate of water loss in thermal profile, we found a positive influence of $T_{\min}$ on the rate of water loss in warm populations ($\beta = 1.74 \pm 0.34$, $t_{1,5} = 5.05$, $p = 0.004$, Fig. 4) and no relationship in cold populations ($t_{1,5} = 0.33$, $p = 0.758$, Fig. 4).

3) Reproductive performance

Population effect

The ED was significantly different between the populations ($F_{9,161} = 16.25$, $p < 0.001$) as well as the ARE ($F_{9,161} = 4.23$, $p < 0.001$) and the RRE ($F_{9,161} = 5.67$, $p < 0.001$).

Correlations with microclimatic covariates

Regarding the correlation between ED and microclimatic covariates, models selection retained the positive influence of T_{mean} , while the null model was retained for ARE and RRE as the dependent variable (Table 3). However, in models testing the effects of $T_{\min}$ and $H_{\min}$, we find marginal effect of the interaction on the ARE ($\beta = -0.07 \pm 0.03$, $F_{1,6} = 3.80$, $p = 0.099$) and significant effect on the RRE ($\beta = -0.08 \pm 0.02$, $F_{1,6} = 16.86$, $p = 0.006$). Specifically, both the ARE and the RRE were negatively correlated with $H_{\min}$ in warm populations (ARE: $\beta = -0.15 \pm 0.08$, $t_{1,6} = -1.96$, $p = 0.097$, RRE: $\beta = -0.13 \pm 0.04$, $t_{1,6} = -3.06$, $p = 0.022$, Fig. 5), while only RRE was positively correlated to $H_{\min}$ in cold population ($\beta = 0.15 \pm 0.04$, $t_{1,168} = 3.91$, $p = 0.008$, Fig. 5).

DISCUSSION

Water is a vital resource often overlooked in ecological studies (Bonnet & Brischoux, 2008). In this study, we investigated the variation of water balance (i.e., hydration state and water loss) in males and females common lizards (*Z. vivipara*) during summer, along microclimatic gradients in temperature and humidity. Contrary to our first prediction, males tended to be more dehydrated than females, while the rate of water loss were similar between those. Then we found sex-specific correlations between the plasma osmolality and/or the rate of water loss and the microclimatic conditions. Overall, we found that minimal conditions in temperature and humidity better correlated with the physiological traits related to water balance and reproductive performance than mean or maximal conditions. Moreover, contrary to our predictions the effects of temperature and humidity were mostly interactive. That is, when comparing cool and warm populations we found different, often opposite correlations between osmolality, water loss, reproductive performance and/or habitat humidity.

Although sex difference in the dehydration rate and/or water loss have already been documented (Stachenfeld *et al.*, 2001; Cryan & Wolf, 2003; Weldon *et al.*, 2013), we would have expected pregnant females to be more dehydrated than males due to physiological changes over pregnancy (Dupoué *et al.*, 2015b). For instance, pregnant or gravid females may experience higher rate of water loss due to higher metabolic rate and transpiration rate (Schultz *et al.*, 2008; Dupoué & Lourdais, 2014; Dupoué *et al.*, 2015a; Webber *et al.*, 2015), the two major determinants of evaporative water losses (Mautz, 1982; Woods & Smith, 2010). Furthermore, developing embryos also need water for somatic growth, and may impact female hydration state (Du, 2004; Dupoué *et al.*, 2015b; Lourdais *et al.*, 2015). However, in our study we cannot distinguish the effect of sex itself from the effect of physiological state (non-breeding *versus* reproductive) since all sampled females were pregnant. Still, we hypothesize the behavior to be one of the first determinant of sex difference in dehydration rate. Compared to males, pregnant females may select lower temperature, and beside stayed relatively inactive as suggested by a previous study (Le Galliard *et al.*, 2003). Those thermoregulatory and activity changes might in turn have lowered the rate of water loss and eventually on the dehydration rate.

When investigating the effects of microclimatic conditions, we found that minimal conditions were better correlates than mean or maximal conditions. Minimal conditions might be more relevant since individuals are necessarily exposed to those conditions and for a relative long period ($> 4\text{h}$), while maximal conditions last for a lower period ($< 1\text{h}$) and can be easily avoided through behavior adjustments such as microclimate selection (Davis & Denardo, 2009; Guillon *et al.*, 2014). Then the interactive effects of temperature and humidity on physiological traits and reproductive performance suggest complex responses to habitat conditions. In males from cool populations, individuals from dryer populations are more dehydrated than ones from humid population, and yet osmolality negatively correlated with the rate of water loss. Conversely, we found opposite relationships in males from warm populations. In females, we did not find any correlation between osmolality or water loss and humidity or with reproductive performance, likely because humidity directly interact with temperature to correlate with the reproductive effort. In females from cool populations, individuals from dryer populations have lower reproductive effort than individuals from humid populations. Again, we found an opposite pattern in females from warm populations. Those opposite correlations of physiological traits or reproductive effort against humidity between cool and warm populations may suggest that individuals of our study adjusted their physiology and/or their behavior before the sampling period. Both humidity (i.e., water vapor pressure) and temperature (i.e., skin resistance, metabolic rate) may impact the rate of water loss (Mautz, 1982; Lillywhite, 2006; Dupoué *et al.*, 2015a) and eventually the dehydration rate, animals may adapt or acclimate to different levels of hydric constraints. In cool environments, the degree of aridity likely represents the only level of constraints so that our results are consistent with predictions on humidity effects. In warm climates however, both temperature and aridity are likely to impact water loss so physiological and behavioral adjustments related to lizard impermeability may prevail over microclimatic effects and induce in turn opposite correlations between osmolality, water loss or reproductive effort and habitat humidity.

Moreover, in males and females, we did not find any correlation between the rate of water loss and humidity inconsistently to our predictions and to previous inter- and intra-specific comparison (Hertz *et al.*, 1979; Tieleman *et al.*, 2003; Williams *et al.*, 2004; Moen *et al.*,

2005; Gunderson *et al.*, 2011; Van Sant *et al.*, 2012; Johnson *et al.*, 2013; Guillon *et al.*, 2014; Cox & Cox, 2015). However, this result needs to be cautiously interpreted because the precision of measures may have not been optimal (i.e., mass variations instead of gas exchanges), and beside in males we only obtained measured of the rate of water loss from 6 populations (3 cool and 3 warm). Even less expected, we found positive relationships between the rate of water loss and temperature in males, and also in females from warm populations. We predicted an opposite pattern since water loss increases with temperature (lower skin resistance and higher metabolic rate) (Webster *et al.*, 1985; Lillywhite, 2006; Dupoué *et al.*, 2015a). However, over the active period, individuals do not only face demand in water resource, since they also have to mobilize energy to perform critical activities (breeding, foraging, etc). In ectotherms, metabolic rate and energy expenditure exponentially increase with ambient temperature (Bennett and Dawson, 1976; and all reference therein). In warmer climates, individuals may increase their use of energy storage (i.e., lipids) to sustain higher metabolic demand. Consequently the lipids available to sustain a skin water barrier may be negatively impacted in warm environments, making individuals more permeable. Indeed, in uricotelic species (e.g., squamate reptiles and birds), the rate of TEWL and especially the rate of transpiration is the main avenue for water losses (Kattan & Lillywhite, 1989; Lillywhite, 2006; Williams *et al.*, 2012), and the keratin-lipids complex (sandwich-like layers) localized in the stratum corneum (i.e., the outer layer of the epidermis) constitutes the main barrier limiting the loss of water (Bouwstra *et al.*, 2003; Lillywhite, 2006; Champagne *et al.*, 2012). The permeability of this water barrier can be adjusted by modifying its thickness (quantity of lipids), the proportion of the different lipids with specific polarity (e.g., cholesterol, fatty acids, phospholipids and ceramides), and/or their geometry (Lillywhite, 2006; Williams *et al.*, 2012). Unfortunately, our study does not allow to distinguish the phenotypic plasticity from the genetic adaptations but rather suggests further investigations. For instance, next studies (e.g., common garden experiments) could specifically determine the variation in the quality of the water barrier by directly measuring the quantity and the types of lipids in ecdysis across populations (i.e., non-invasive sampling methods), while the lipid disposition could be assessed for example with electronic microscopy.

Although correlative, comparative studies offer useful opportunity in understanding and predicting how far species can adapt or acclimate to climatic conditions (Somero, 2011; Rezende & Diniz-Filho, 2012). Water resource is an essential resource and paradoxically,

relatively overlooked in ecological studies. Yet, water availability is expected to change in future climates (IPCC 2014) and predicting species responses to dehydration and loss of water have never been as important. As demonstrated here, responses to water availability can be multiple, interactive and overall complex, and we thereby need more integrative and experimental studies (Todgham & Stillman, 2013). For instance, further studies should specifically examine the relative contribution from physiological adaptation or acclimation to behavioral mitigation in adjusting the rate of water loss and eventually the rate of dehydration. Such information are essential to improve the accuracy of models predicting species responses to climate change and promote adequate conservation measures (Wikelski & Cooke, 2006; Cooke *et al.*, 2013).

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Table 1. Microclimatic conditions in the studied populations of common lizard (*Z. vivipara*) and sample size of males and females caught in each population for each physiological measure. Table shows the mean $\pm$ SE of habitat temperature and humidity (mean: T_{mean} and H_{mean} , minimal: T_{min} and H_{min} , and maximal: T_{max} and H_{max}) recorded between the 29th June and the 17th July 2015. Population thermal profile was defined based on the minimal temperature (cool: $T_{\text{min}} < 9.5^{\circ}\text{C}$, warm: $T_{\text{min}} > 9.5^{\circ}\text{C}$). See text for details.

		Microclimatic conditions						Thermal profile	Physiological measure			
		Temperature (°C)			Humidity (g.m ⁻³)				Osmolality		Water loss	
Population	Elevation (m)	<i>T</i> _{mean}	<i>T</i> _{min}	<i>T</i> _{max}	<i>H</i> _{mean}	<i>H</i> _{min}	<i>H</i> _{max}		Males	Females	Males	Females
MON	1099	20.39 ± 0.31	11.42 ± 0.42	33.13 ± 0.32	16.37 ± 0.33	9.45 ± 0.52	26.63 ± 0.48	Warm	13	7	7	16
VIA	1191	19.06 ± 0.32	8.53 ± 0.52	35.45 ± 0.58	-	-	-	Cool	12	18	11	24
PEJ	1254	20.39 ± 0.64	8.00 ± 0.95	39.19 ± 1.04	13.41 ± 0.60	7.44 ± 0.44	24.46 ± 1.15	Cool	9	21	-	-
CARM	1268	20.58 ± 0.62	9.68 ± 0.92	32.97 ± 0.79	-	-	-	Warm	9	24	10	24
JOC	1282	19.09 ± 0.30	8.77 ± 0.53	35.78 ± 0.61	12.78 ± 0.32	7.81 ± 0.26	24.20 ± 0.79	Cool	7	24	10	25
LAJ	1383	20.72 ± 0.47	8.79 ± 0.46	39.04 ± 0.66	11.99 ± 0.32	7.53 ± 0.25	19.10 ± 0.45	Cool	8	22	8	23
JON	1385	18.42 ± 0.38	10.06 ± 0.43	33.73 ± 0.68	13.56 ± 0.33	9.74 ± 0.26	22.53 ± 0.51	Warm	6	22	10	28
CHA	1416	19.96 ± 0.51	10.49 ± 0.77	35.63 ± 0.68	11.61 ± 0.37	7.85 ± 0.32	19.13 ± 0.82	Warm	10	12	-	14
COP	1421	20.36 ± 0.33	7.16 ± 0.43	44.74 ± 0.77	-	-	-	Cool	5	14	-	15
COM	1454	18.97 ± 0.51	10.20 ± 0.61	29.93 ± 0.68	12.30 ± 0.44	7.92 ± 0.32	19.92 ± 0.70	Warm	7	14	-	14
BEL	1464	18.10 ± 0.45	8.80 ± 0.56	33.96 ± 0.49	16.38 ± 0.49	9.64 ± 0.38	32.65 ± 1.06	Cool	10	24	10	25
PUY	1470	19.98 ± 0.51	12.63 ± 0.60	32.55 ± 0.84	14.29 ± 0.40	10.51 ± 0.34	21.75 ± 0.69	Warm	13	10	14	20
BARN	1531	20.58 ± 0.39	9.04 ± 0.62	38.50 ± 0.62	16.64 ± 0.32	9.29 ± 0.33	28.35 ± 0.53	Cool	9	15	-	14

Table 2. Model selection comparing the influence of microclimatic variables and reproductive performance on the plasma osmolality and the rate of water loss in males and females. Population was treated as a random factor to account for intra-population variation and non-independence.

Dependent variable	Males		Females	
	Model	k AIC	Model	k AIC
Osmolality	~1	3 881.8463	~1	3 1693.261
	T_{mean}	4 875.0521	T_{mean}	4 1690.489
	H_{mean}	4 879.5095	H_{mean}	4 1691.168
	$T_{\text{mean}} + H_{\text{mean}}$	5 872.8612	$T_{\text{mean}} + H_{\text{mean}}$	5 1688.351
	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6 869.8972	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6 1684.821
	T_{min}	4 878.9315	T_{min}	4 1690.235
	H_{min}	4 878.4382	H_{min}	4 1690.345
	$T_{\text{min}} + H_{\text{min}}$	5 874.9371	$T_{\text{min}} + H_{\text{min}}$	5 1687.296
	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6 867.0070	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6 1683.130
	T_{max}	4 881.1824	T_{max}	4 1692.752
	H_{max}	4 881.1813	H_{max}	4 1693.349
	$T_{\text{max}} + H_{\text{max}}$	5 877.7254	$T_{\text{max}} + H_{\text{max}}$	5 1692.751
	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6 878.1977	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6 1694.480
	-	- -	ED	4 1688.719
	-	- -	ARE	4 1688.671
	-	- -	RRE	4 1693.462
	-	- -	ED + ARE	5 1688.465
	-	- -	ED + RRE	5 1688.874
	-	- -	ED * ARE	6 1687.700
	-	- -	ED * RRE	6 1686.278
Water loss	~1	3 316.0089	~1	3 899.6654
	T_{mean}	4 314.6652	T_{mean}	4 900.8311
	H_{mean}	4 315.2595	H_{mean}	4 901.1414
	$T_{\text{mean}} + H_{\text{mean}}$	5 312.0478	$T_{\text{mean}} + H_{\text{mean}}$	5 902.2836
	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6 312.8757	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6 904.6836
	T_{min}	4 311.8799	T_{min}	4 899.5606
	H_{min}	4 314.1751	H_{min}	4 898.9151
	$T_{\text{min}} + H_{\text{min}}$	5 311.5931	$T_{\text{min}} + H_{\text{min}}$	5 900.1776
	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6 311.3524	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6 900.8758
	T_{max}	4 315.3499	T_{max}	4 902.8794
	H_{max}	4 318.3459	H_{max}	4 903.2512
	$T_{\text{max}} + H_{\text{max}}$	5 317.7108	$T_{\text{max}} + H_{\text{max}}$	5 906.4214
	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6 319.1633	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6 912.5138
	-	- -	ED	4 901.1973
	-	- -	ARE	4 900.5918
	-	- -	RRE	4 905.5556
	-	- -	ED + ARE	5 906.9271
	-	- -	ED + RRE	5 906.4673
	-	- -	ED * ARE	6 910.7655
	-	- -	ED * RRE	6 908.1972

Table 3. Model selection comparing the influence of microclimatic variables on the reproductive performance (Embryonic development, ED: estimated as the days before parturition; absolute reproductive effort, ARE: estimated as the mass of neonates; relative reproductive effort, RRE: derived from the relationship between litter mass and SVL). Population was treated as a random factor to account for intra-population variation and non-independence.

Dependent variable	Model	k	AIC
ED	~1	3	918.3324
	T_{mean}	4	914.3374
	H_{mean}	4	919.7373
	$T_{\text{mean}} + H_{\text{mean}}$	5	916.0201
	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6	917.1478
	T_{min}	4	919.1293
	H_{min}	4	918.4138
	$T_{\text{min}} + H_{\text{min}}$	5	918.6017
	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6	915.0307
	T_{max}	4	919.5938
	H_{max}	4	921.3869
	$T_{\text{max}} + H_{\text{max}}$	5	922.6405
	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6	926.1674
ARE	~1	3	188.3796
	T_{mean}	4	190.3037
	H_{mean}	4	194.9644
	$T_{\text{mean}} + H_{\text{mean}}$	5	197.3254
	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6	204.0531
	T_{min}	4	191.4800
	H_{min}	4	193.5475
	$T_{\text{min}} + H_{\text{min}}$	5	196.9258
	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6	200.3839
	T_{max}	4	196.1310
	H_{max}	4	194.1052
	$T_{\text{max}} + H_{\text{max}}$	5	202.0777
	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6	212.3481
RRE	~1	3	108.8909
	T_{mean}	4	113.1383
	H_{mean}	4	114.6871
	$T_{\text{mean}} + H_{\text{mean}}$	5	119.1870
	$T_{\text{mean}} + H_{\text{mean}} + T_{\text{mean}} \times H_{\text{mean}}$	6	125.8478
	T_{min}	4	111.4131
	H_{min}	4	114.5030
	$T_{\text{min}} + H_{\text{min}}$	5	116.4088
	$T_{\text{min}} + H_{\text{min}} + T_{\text{min}} \times H_{\text{min}}$	6	114.4906
	T_{max}	4	116.6389
	H_{max}	4	113.5289
	$T_{\text{max}} + H_{\text{max}}$	5	121.7673
	$T_{\text{max}} + H_{\text{max}} + T_{\text{max}} \times H_{\text{max}}$	6	132.1199

Figure caption

Figure 1. Localizations of the studied populations of common lizard (*Z. vivipara*) in the Massif Central, France. Exact coordinates of populations are as followed: BARN (Barnessac, 44°25'27.35"N; 3°45'53.80"E), BEL (Bel Air, 44°40'20.79"N; 4° 1'29.84"E), CARM (Carmantran, 45° 9'27.99"N; 2°50'16.71"E), CHA (Chalet du Mont Lozère, 44°26'56.72"N; 3°44'51.79"E), COM (Col du cheval Mort, 44°40'1.29"N; 3°31'57.98"E), COP (Col du Pendu, 44°39'16.75"N; 4° 1'51.48"E), JOC (Gerbier de Jonc 2, 44°50'6.66"N; 4°12'39.65"E), JON (Gerbier de Jonc 1, 44°50'30.31"N; 4°12'54.30"E), LAJ (Lajo, 44°50'41.10"N; 3°25'51.01"E), MON (Montselgues, 44°30'36.36"N; 4° 0'26.03"E), PEJ (Pejouzou, 45° 9'49.07"N; 2°50'39.72"E), PUY (Puy Mary, 45°6'25.77"N; 2°41'6.10"E) and VIA (44°20'16.59"N; 3°46'7.35"E).

Figure 2. Relationships between plasma osmolality and habitat humidity ($H_{\min}$) in males (n = 92) and females (n = 171) from cool (open circles) or warm (full circles) populations of common lizards. Trend lines (dashed line: cool populations, solid line: warm populations) are included for significant relationships.

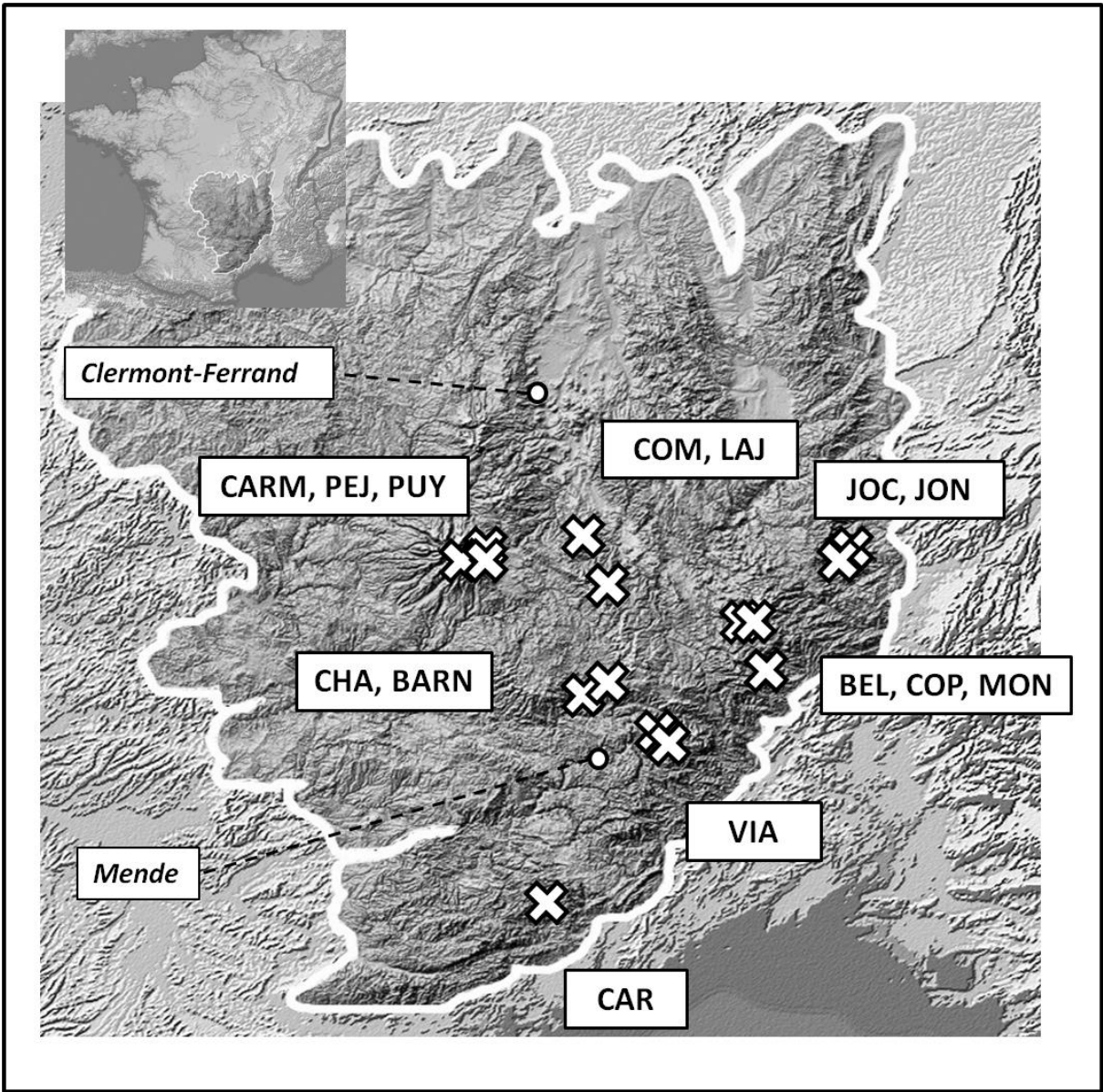
Figure 3. Relationships between plasma osmolality and the rate of water loss in males (n = 61) and females (n = 225) from the cool (open circles) and warm (filled circles) populations. Trend lines are included for significant relationships.

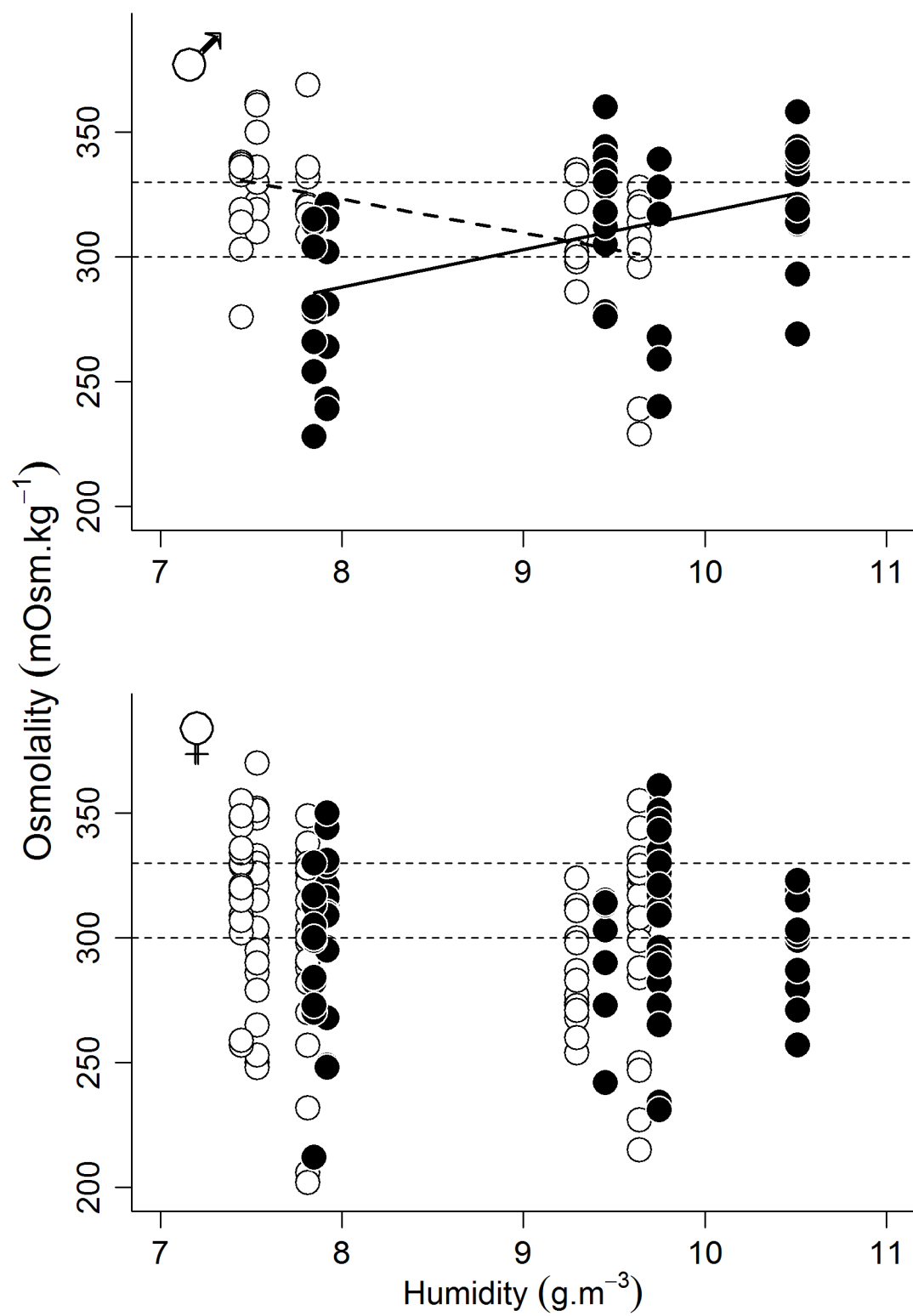
Figure 4. Relationships between the rate of water loss and the microclimatic conditions ($T_{\min}$ and $H_{\min}$) in males ($n = 59$) and females ($n = 179$) from cool (open circles) or warm (filled circles) populations. Trend lines are included for significant correlation.

Figure 5. Relationships between the relative reproductive effort (RRE, derived from the relationship between litter mass and SVL) and humidity ($H_{\min}$) in females from the cool ($n = 106$, open circles) and warm ($n = 65$, filled circles) populations. Trend lines are included for significant relationships.

47 Figure 1.

48

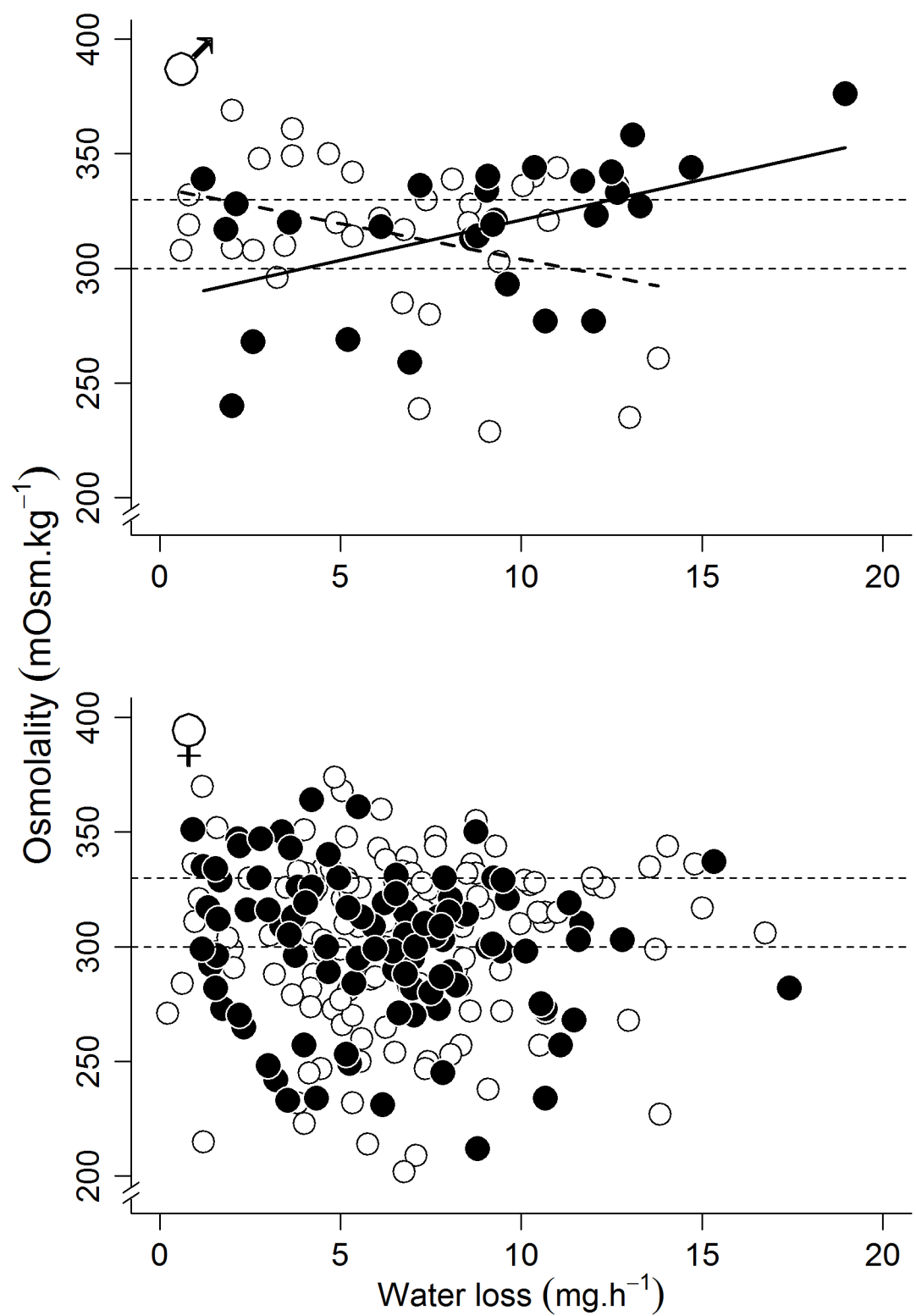




49 Figure 2.

50

Figure 3.



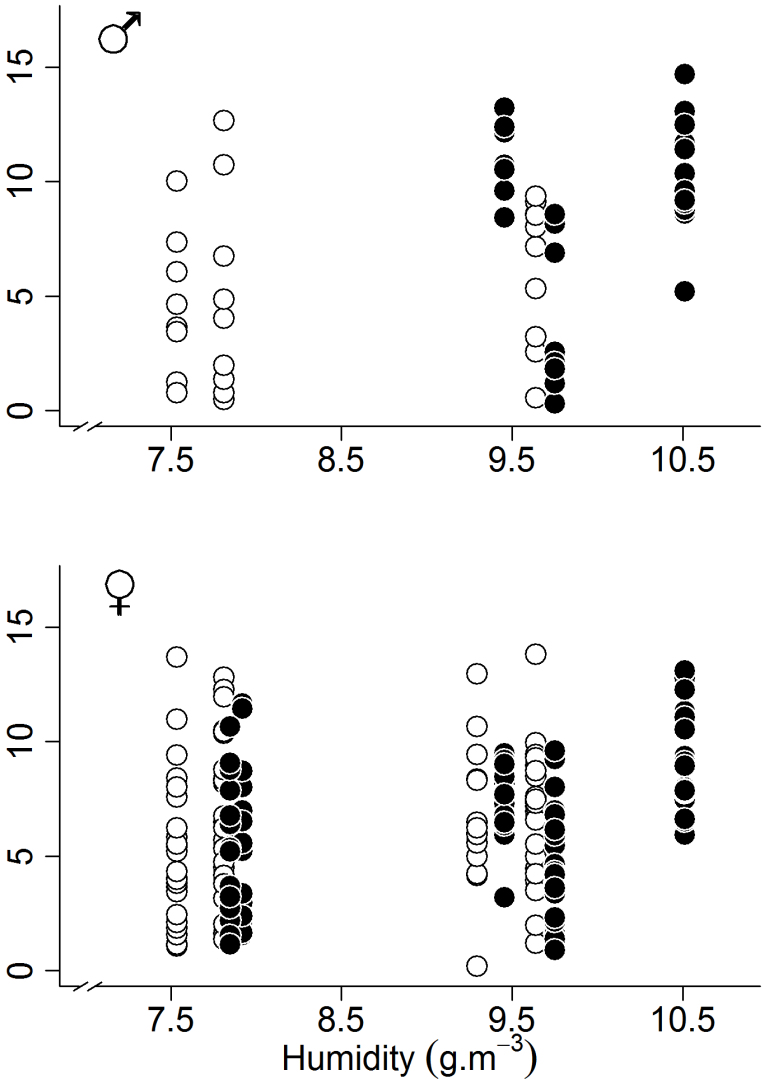
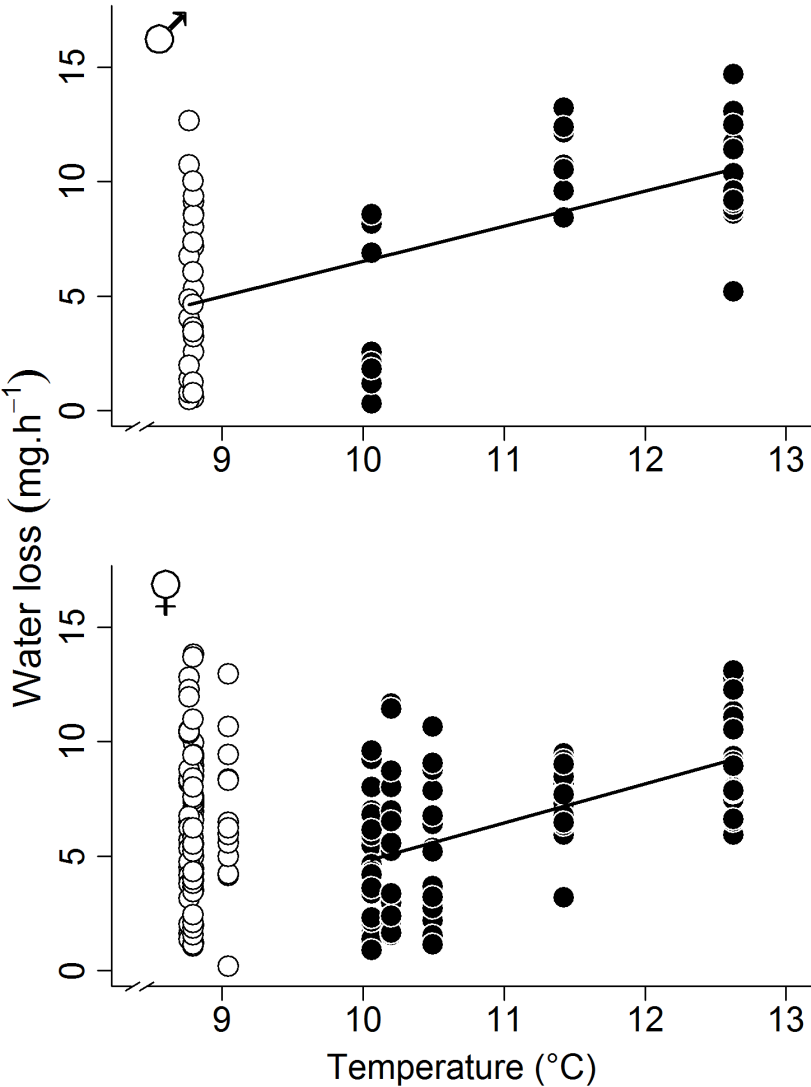
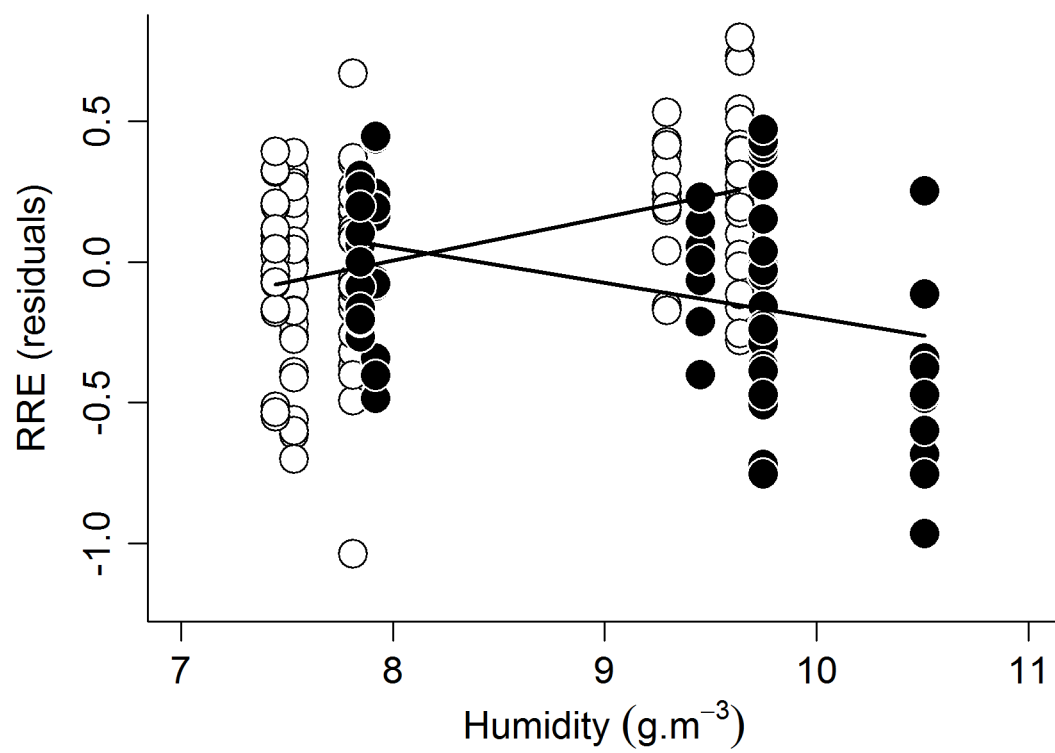


Figure 5.



CHAPITRE A2: Impact de l'environnement sur la charge parasitaire.

Ce chapitre est né d'une idée que je n'ai pas eu le temps de développer au cours de ma thèse et que j'ai donc réalisé avec Qiang Wu, nouveau doctorant de l'équipe. Comme je l'ai déjà décrit, les populations du suivi Global Change sont réparties le long d'un double gradient thermique et anthropique. Nous avons voulu vérifier ici si les animaux d'élevage (ovins, bovins, équins) cohabitant avec le lézard vivipare n'étaient pas vecteurs de parasites (tiques et/ou acariens) et à quel point les conditions climatiques et paysagères affectaient leur transmission. Dans les populations parasitées, nous avons aussi voulu estimer l'impact de la charge parasitaire sur la reproduction.

Les résultats démontrent que la réponse de chacun des parasites répond à des exigences écologiques différentes. Nous avons aussi pu démontrer des effets de chacun des deux parasite sur différents éléments de la reproduction.

Article 5 : Parasites load and co-occurrence under environmental mediation: their influence on reproduction in common lizard *Zootoca vivipara*.

For consideration in: ?, *in progress*

Authors : Q. Wu, A. Rutschmann, D.B. Miles M. Richard & J. Clobert

Abstract : Parasites have significant effects on host fitness by affecting host body condition and behavior, shaping host reproductive investment, mediating tradeoff between life history traits, and even regulating population dynamics. On the other hand, hosts may also have different vulnerability and different adjustment in their response to different parasites. However, one cannot reduce the study of host-parasite relations to their arms race as both biotic and abiotic factor can influence their coevolution. In this study, we examined the effects of two ectoparasites (mite and tick) on the common lizard *Zootoca vivipara*. We first investigated whether parasite load was affected by environmental factors, which included altitude, temperature, habitat cover, human activity and livestock grazing; second we explored potential competition between mites and ticks; finally we focused on the influences of mites and ticks on reproductive outputs and offspring morphology of their host. We found that: (1) mite presence on host was correlated with environmental factors and lizard individual phenotype whereas mite abundance was only related to lizard individual phenotype. On the other hand, tick presence was associated with environmental factors but tick abundance was neither correlated with environmental factors nor individual phenotype. These results suggested that prevalence of the two parasites are affected by distinct patterns of environmental factors, which may indicate mites and ticks have different ecologies; (2) there was no supportive evidence for the existence of co-occurrence between mites and ticks; (3) mite presence was negatively correlated with relative clutch mass but positively with hatchling success, whereas ticks load was negatively associated with laying date. Both mites and ticks showed no effects on offspring morphology. Our study emphasizes the importance of considering environmental factors into the research of coevolution of parasite and host.

INTRODUCTION

Parasites are defined as “organisms that obtain nutrients from one or a very few host individuals” (Begon, Townsend et al. 2006) and play a crucial role in the coevolution process with their hosts (Hamilton 1980). Parasites could influence many elements of the host phenotypes, including behavior, physiology, life history, sexual selection and population dynamics (Sheldon and Verhulst 1996, Schmid-Hempel 2011). However, even if parasites have long been put in the central position of host-parasite coevolution research (Sheldon and Verhulst 1996), one should not ignore the fact that external abiotic factors such as habitat type, temperature, humidity, topography (Tälleklint-Eisen and Eisen 1999, Sehgal, Buermann et al. 2011), and biotic factors such as competition for host (Paul, Nu et al. 2002) or even human activity (Altizer, Harvell et al. 2003) are also expected to affect this coevolution process.

Parasite load could be affected by diverse abiotic factors. For instance, classical environmental factors like altitude (Spoecker 1967), seasonal change (Klukowski and Nelson 2001, Lumbad, Vredevoe et al. 2011), temperature (Pollock, Gawne et al. 2015), vegetation composition (Tälleklint-Eisen and Eisen 1999, Eisen, Eisen et al. 2004), refuge type used by host (Kerr and Bull 2006), microhabitat selection of parasite (Choe and Kim 1988) and distributions of resources (Garrido and Perez-Mellado 2013) are all well known to influence host distribution and patterns of parasitism. In the framework of global change, several factors mediated by human have also been shown to influence the relationship between host and parasite. For example, anti-predatory behavior induced by increasing human activity may comprise the defense against parasite, and enhance the risk of infestation (Amo, López et al. 2007, Garrido and Pérez-Mellado 2015). In addition, habitat deterioration caused by grazing (e.g. fewer food resource, change of microhabitats and reduce of refuges), and more importantly, parasites brought by livestock, can have great impacts on parasite-host relationships (Pettigrew and Bull 2011, Pafilis, Anastasiou et al. 2013). Finally, climate change incurs ecological perturbations, which could cause geographical and phenological changes (e.g. extended distribution range of parasites, increased winter activity of parasite etc.) as well as influences on parasite development, survival rate, host susceptibility and transmission dynamics (Harvell, Mitchell et al. 2002, Brooks and Hoberg 2007, Gray, Dautel et al. 2009). All these are potential factors that need to be considered when analyzing the relationship of parasite and host in the foreseeable decades.

Usually, ectoparasites have negative effects on their host fitness (Lehmann 1993). One important life history trait being most often affected by parasite is reproduction (Engelstädter and Hurst 2009). For example, ectoparasites could influence host mortality (Sorci and Clobert 1995) and long-term survival (Brown, Brown et al. 1995), increase the cost of reproduction (Fitze, Tschirren et al. 2004) and reduce reproductive success (Oppliger, Richner et al. 1994), mediate the tradeoff between clutch size and offspring quality (Saino, Ferrari et al. 2002) or between current and future reproduction (Fitze, Tschirren et al. 2004).

Hosts are seldom infected by only one parasite (Rigaud, Perrot-Minnot et al. 2010), therefore it is a common phenomenon that hosts are co-infected by multiple species of parasites (Pedersen and Fenton 2007). One problem arising with multiple infections is the likely competition among parasites (Brown, Hochberg et al. 2002). Since a host is a limited resource (Ebert and Weisser 1997), it is therefore expected that some strong interspecific competition occurs between parasites sharing the host. However, the competition among ectoparasites has rarely been testified (Baer-Lehman, Light et al. 2012), and few studies try to elucidate different effects of environmental factors on mediating mites and ticks infestation, and how they distinctly affect the reproduction of host.

Among all the ectoparasites, mites and ticks are two common species which parasitize reptiles (Cooper and Jackson 1981). In lizards, plenty of researches have already explored the effects of mites and ticks on lizard fitness, behavior, thermoregulation etc. (Bull and Burzacott 1993, Sorci, Massot et al. 1994, Sorci and Clobert 1995, Main and Bull 2000, Conover, Cook et al. 2015). The common lizard (*Zootoca vivipara*) is a species which is parasitized by both mites (genus *Ophionyssus*) and ticks (*Ixodes ricinus*), thus providing an excellent study system to study the potential competition between ectoparasites and its environmental mediation. Several researches have already been conducted on the influence of parasites on this species, e.g. effects of environmental stress on blood parasite infection (Oppliger, Clobert et al. 1998), effects of maternal parasite load on offspring life-history traits (Sorci and Clobert 1995), parasitism and host reproductive effort (Sorci, Clobert et al. 1996) and host density-dependence infestation (Mugabo, Perret et al. 2015). But the knowledge about different effects of mites and ticks on the common lizard infestation is still lacking.

In this study, we aim to investigate the environmental factors which may affect parasites infestation, the potential existence of competition between mites and ticks and the influence of parasites on host reproduction. For this purpose, we collected data on lizard parasites in twelve populations on a altitudinal (temperature) and man induced perturbation. We then

searched for the effect of altitude and disturbance on the respective mites and ticks density on host, and their effect on host reproductive success.

MATERIAL AND METHODS

The Host

The common lizard (*Zootoca vivipara*) is a small viviparous lizard (adult snout-vent length varies from 40 to 60 mm in males and 45 to 75 mm in females), which is widely distributed in Europe and north Asia (Pilroge 1987). It usually inhabits peat bogs, heath land and is found in most humid habitats. Mating starts in early May immediately after females emergence from hibernation (from end October to early May), with parturition coming two months later (Sorci and Clobert 1995), early June nowadays (Chamaillé-Jammes, Massot et al. 2006).

The Parasite

The two ectoparasites we concentrated on were the haematophagous mite which belongs to the genus *Ophionyssus* (Sorci, Fraipont et al. 1997) and the sheep tick *Ixodes ricinus*. The mites are temporary parasites living freely in the soil and climbing on host for engorging blood (Cooper and Jackson 1981). Ticks are mainly brought by large animals (Gray, Dautel et al. 2009). Both these two ectoparasites could serve as vectors for transmitting blood parasites or disease (Cooper and Jackson 1981, Gray 1991).

Lizard sampling and parasite collection

The samplings of lizards were performed in 2012, 2014 and 2015, usually from 15th June till early July from 12 different sites in the Massif Central, southern France. 15 to 25 females and 10 males were captured in every population and brought back to a field laboratory. Immediately back to the laboratory facilities, all lizards were weighed and measured (Snout-ventral length, SVL). Each individual was visually examined for ectoparasite (mites and ticks) infestation, and then parasites were removed and kept in a tube with 95% alcohol for further identification. The period of embryonic development which is most sensitive to environmental condition for viviparous reptiles is the middle part of the gestation (Lourdais, Shine et al. 2004 in the viviparous snake *Vipera aspis*). We assume therefore that the impact of parasites was already maximum when we started to collect pregnant females in the field.

After removing all the ectoparasites, lizards were treated with a 0.1% trichlorfon solution to make sure they will not carry parasites any more in the lab (Sorci, Massot et al. 1994). The

number of mites and ticks was recorded for every lizard. Then, lizards were individually housed in a plastic terrarium (11x18x11 cm) with damp soil and an egg box as a shelter. They received six hours of light everyday for thermoregulation and were fed with crickets. At parturition, we recorded the clutch size and every juvenile within the clutch was measured and weighted to assess the quality of the reproduction. Three days after parturition, each female was released with her offspring at the exact capture location.

Statistical analysis

Parasite load and competition between parasites

We used Mann-Whitney U tests to compare mite and tick abundance between host sexes. Mites and ticks abundance were compared using Wilcoxon signed rank tests, and the overall association of mites and ticks abundance was tested by using Kendall's tau rank correlations. Note that when analyzing the association, we excluded the lizards which have zero mites and zero ticks to decrease the effect of excess zeroes. We used generalized linear mixed effects model (Bolker, Brooks et al. 2009) to analyse the relationship between mites and ticks, which tick was included as the dependent variable while mites, environmental factors and their interactions as the independent factors. The site effect was included as a random effect. After that, we conduct a selection for fixed factors by using glmmLasso package.

Influence of habitat on parasite load

All the environmental factors in study sites were measured following Rutschmann, Miles et al. (2015). We conducted a principal components analysis (PCA) on five environmental factors by using psych R package. These factors include altitude, maximum temperature in June, habitat cover, human activity and grazing activity (0 no grazing, 1 grazing). We chose the first two PCA axes to represent environmental factors. Mites and ticks presence (Binomial distribution) were examined with generalized linear model, which included two PCA axes describing environment, the sex, SVL and weight of the host (we didn't report the results of model averaging which include $SVL \times Weight$ because this effect almost never appeared in the confidence model set). The abundance of mites and ticks (Poisson distribution) was examined by generalized linear model with the five same factors as above. We used maximum likelihood approach to fit models on our data. As mite and tick abundance models both showed significant overdispersion (ratio between residual deviance and residual degrees of freedom, $\hat{c}=3.74$ and 1.30 respectively), we used GLM with the correction for overdispersion. Negative binomial model is an alternative method for correcting

overdispersion, but we obtained similar results, so only the results of the poisson model were reported in this study.

We used Akaike's information criterion (AIC) for model selection for mite and tick presence (AIC is used as the sample size is large enough, $n=776$). QAICc was used for model selection for mite and tick presence ($n=222$ and 229 respectively) by correcting for overdispersion. We used Akaike weights w_i as the measure of the relative probability of one model being chosen as the expected best model. w_i is calculated following Burnham and Anderson (2002). When several models have close AIC values or plausibility, we conducted model averaging by using MuMIn R package as the following steps:

(1) Confidence set of the models. We chose the criterion of 95% confidence set for models by following the decreasing order of model weight w_i until the cumulative weights first reaches 0.95 (Burnham and Anderson 2002).

(2) Estimating the relative importance of predictable variables. We estimated the relative importance of variables by accumulating the Akaike weights of all the models in the set where that variable appears (Burnham and Anderson 2002).

(3) Estimating the predict parameter. We estimated the model-averaged parameter based on Akaike weights by averaging the parameter across all the models in which that parameter exists. (Burnham and Anderson 2002).

Influence of parasite on host reproductive efforts and offspring morphology

Effects of the parasite load on clutch size, hatchling success and relative clutch mass were examined by using generalized linear model. Parasite effects on parturition date were inspected by linear regression, and parturition date was defined as the day number in the whole year. As mites and ticks abundance are zero-inflated variables, we first used parasite presence and then used zero truncated parasite abundance in the regression analysis. For the influence of parasite load on offspring size (e.g. body mass, snout-vent length and total body length), we used linear mixed effects model which parasite load, PC1, PC2 as fixed factors and family appearance as a random factor.

All the analyses were conducted in R 3.1.3.

RESULTS

Prevalence

A total of 776 lizards have been examined for mites and ticks in 2012 ($n=286$, 165 females and 121 males), 2014 ($n=267$, 179 females and 88 males) and 2015 ($n=223$, 170

females and 53 males). Among 776 lizards, 31(3.99%) were co-infested by both mites and ticks, 136 (17.53%) only had mites and 91 (11.73%) only had ticks, 518 (66.75%) had neither mites nor ticks. The overall prevalence of parasites was 33.25%, with mites 21.52% and ticks 15.72%.

Mites ($U=58181.5$, $P<0.001$), but not ticks ($U=65120.5$, $P=0.2366$) were more abundant in males than females, also mite was more abundant than ticks overall ($W=20799.5$, $P<0.001$). Meanwhile we detected an overall negative correlation between mites and ticks abundance ($n=258$, $\tau = -0.536$, $P<0.001$).

Influence of habitat on parasite load

The first and second axes of PCA (Table 1) accounted for 74% of all environmental factors variation. PC1 is strongly correlated with four of the original variables: altitude, cover, human activity and grazing. PC1 increases with altitude, habitat cover, and decreases with human activity and grazing. Therefore, we explain PC1 as the index of habitat quality and anthropogenic gradient factor. PC2 is only correlated with temperature, and increases with max temperature in June, thus we refer PC2 as the thermal factor.

Table 1. The main results of principal component analysis

	First component(PC1)	Second component(PC2)
Eigenvalue	2.55	1.13
Proportion Variation	0.51	0.23
Cumulative Variation	0.51	0.74
Altitude	0.82	0.00
Tmax6	0.22	0.90
Cover	0.79	-0.22
Human activity	-0.85	0.36
Grazing	-0.70	-0.40

Based on the criterion of summing model weights ≤ 0.95 , there are 10 and 23 models in the confidence set for explaining mite presence and abundance respectively (Appendix A: Table 1 and 3). The sex effect was present in all models for explaining mite presence. As for the model explaining tick, there are 7 and 25 candidate models in confidence set for predicting tick presence and abundance respectively (Appendix A: Table 2 and 4). PC1 and PC2 were present in all the models for explaining the tick presence. The model averaging

presents us the relative importance of PC1, PC2 and individual phenotype of lizards for predicting the presence and abundance of two kinds of parasite respectively (see Table 2).

Our results showed: (1) The mite presence was positively correlated with PC1, sex being male, SVL, and negatively correlated with the weight of lizard. Thus, lizard with longer SVL, lower weight, sex being male, inhabiting at high altitude and in habitats with a higher plant cover (consequently with low human activity and grazing activity), should have a higher chance of being infested by mites. Meanwhile the abundance of mite was however only positively correlated with sex being male, SVL and negatively correlated with body weight (i.e. only correlated with lizard individual phenotype). Thus, lizards with longer SVL, lower weight and sex being male were easily infested by a greater number of mites (Table 2). (2) The tick presence was negatively correlated with PC1 and PC2. We can therefore concluded that lizards which inhabited in habitats with high human activity and grazing activity (consequently with low altitude and plant cover) and low temperature, were more susceptible to tick infestation. However, there weren't any significant factors for explaining tick abundance (Table 2).

Table 2. Relative importance and model weighted averaging parameter coefficient estimates for presence and abundance of two parasites. Abbreviation, IM means importance; COEF means coefficient; SE means standard error.

	presence of mites			abundance of mites			presence of ticks			abundance of ticks		
	IM	COEF	SE	IM	COEF	SE	IM	COEF	SE	IM	COEF	SE
PC1	0.8	0.2059*	0.0925	0.28	0.0488	0.0402	0.93	-0.5570***	0.1005	0.5	-0.1107	0.066
PC2	0.24	-0.0195	0.0917	0.27	-0.0571	0.0450	0.93	-0.3094**	0.1054	0.37	-0.0756	0.056
Sexm	0.95	0.8508***	0.2414	0.66	0.3609*	0.1139	0.47	0.3412	0.2294	0.24	0.0658	0.131
SVL	0.77	0.0590*	0.0287	0.62	0.0487*	0.0176	0.24	-0.0037	0.0185	0.26	-0.0075	0.012
Weight	0.7	-0.2784*	0.1361	0.77	-0.2670*	0.1014	0.25	-0.0441	0.1001	0.27	-0.0482	0.064

*0.05>P>0.01,**0.01>P>0.001,***P<0.001

Co-occurrence between ticks and mites

Using generalized linear mixed effects model, with the sample sites as random effects and after the selection for fixed factors, we detected mite and the interaction between mite and PC1 as two significant terms (Table 3). By using this optimal model, we found a weak significant negative relationship between ticks and mites (Table 4).

Table 3. Coefficients of GLMM model for fixed factors selection

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-0.259617	0.323510	-0.8025	0.4223
MITE	-0.224853	0.055129	-4.0786	4.530e-05 ***
PC1	-0.024288	0.301854	-0.0805	0.9359
PC2	0.037826	0.299642	0.1262	0.8995
MITE:PC1	-0.156948	0.038193	-4.1093	3.969e-05 ***
MITE:PC2	-0.041943	0.031100	-1.3487	0.1774

*0.05>P>0.01, **0.01>P>0.001, ***P<0.001

Table 4. Coefficients of GLMM model for relationship between ticks and mites

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-0.30966	0.28704	-1.079	0.281
MITE	-0.20375	0.04864	-4.189	2.80e-05 ***
MITE:PC1	-0.14091	0.03311	-4.256	2.08e-05 ***

*0.05>P>0.01, **0.01>P>0.001, ***P<0.001

Table 5 Influence of parasite load on reproductive investment and offspring morphology

	Mite presence	Mite load(zero truncated)	Tick presence	Tick load(zero truncated)
Clutch size	no	no	no	no
Hatchling success	+	no	no	no
Relative clutch mass	-	no	no	no
Laying date	no	no	-	-
Offspring body mass	no	no	no	no
Offspring snout-vent length	no	no	no	no
Offspring total body length	no	no	no	no

Impact of parasite load on reproductive effort and offspring morphology

Clutch size was not correlated with parasites load for both mites and ticks, but positively associated with female snout-vent length (Appendix B: Table 1-4). Mite presence was positively correlated with hatchling success (Appendix B: Table 5) and negatively associated with Relative clutch mass (Appendix B: Table 9). Tick presence and abundance were both negatively correlated with parturition date (Appendix B: Table 15 and 16). As for offspring size, snout-vent length, total body length and body mass were all not correlated with parasites loads (Appendix B: Table 17 to 32). See Table 5 for the simple summarizing results of effects of parasite load on reproductive investment and offspring morphology.

Table 5 Influence of parasite load on reproductive investment and offspring morphology

DISCUSSION

In this study, we investigated the tenets of presence and abundance of two ectoparasites on the common lizard under different environmental factors. We showed that mite presence was affected by both environmental factors and lizard individual phenotype, while mite load was only correlated with lizard individual phenotype. In contrast, tick presence was only affected by environmental factors but somehow with a different pattern comparing to mite presence, and tick load was unrelated to both environmental factors and individual phenotype. Furthermore, there was no clear co-occurrence between mites and ticks. Finally, we found that mite presence was negatively correlated with the relative clutch mass but positively associated with hatchling success, whereas tick load correlated negatively with female laying date. Both mites and ticks had no influence on clutch size and offspring morphology.

Influence of habitat factors on parasite load

Our results suggest that both mites and ticks presence are influenced by environmental factors, but with different trends according to the parasite. Higher presence and load of mite were correlated positively with altitude and cover intensity, but decreased with human activity and grazing. On the contrary, higher presence of tick was associated with higher levels of grazing and human activity, as well as lower altitude, cover and temperature. This may suggest that these two kinds of ectoparasites have different ecologies.

Mite load increased with elevation, and results obtained from previous researches either support or go against this hypothesis (Spoecker 1967, Zippel, Powell et al. 1996, Carothers and Jaksic 2001). Spoecker (1967) and Zippel, Powell et al. (1996) indicated that the pattern

of mite load increasing with elevation might result from the milder humid surroundings at higher altitude habitats, rather than elevation *per se*. Unfortunately, we did not have the data on habitat humidity to disentangle humidity from elevation on mite load. Another explanation relies on positive relationship between mite presence and better plant cover. As plants could provide preferable sites for lizards' perching, basking and ambush foraging (Garrido and Perez-Mellado 2013), thus higher plant cover sites could serve as preferred habitats for lizards. Garrido and Perez-Mellado (2013) indicated that increased parasitism in abundant plant areas may be due to the higher density of lizards, because lizards inhabiting in preferred micro-habitats will face a heavy social stress and that could in turn reduce the efficiency of immune system (Oppliger, Clobert et al. 1998). On the other hand and based on the observation that mite load is higher at mesic and densely forest sites, which may suggest humidity and protective cover play important role in affecting mite abundance rather than competition affecting lizard resistance to parasites (Reed 2014). Again without knowing host density and parasite habitat preference, these explanations need to be further tested in our population. At the same time, there is a negative relationship between tick presence and altitude as well as plant cover. This negative association along altitudes may due to the temperature effect which will limit the development rates of ticks (Jouda, Perret et al. 2004, Randolph 2004). It is a bit surprising that vegetation cover has negative effects on tick presence because of its importance for tick survival and development (Lindgren, Tälleklint et al. 2000). But we should notice that different variables may be associated with each other, kind like altitudes, host density, vegetation cover etc., therefore it will be difficult and unwise to tease apart these traits and focus on only one variable for revealing the pattern of parasite prevalence (Gilbert 2010).

Anthropogenic activities also have strong effects on host-parasite relationship. Vegetation alternation induced by grazing can result in a change of microhabitat structure and shelter availability for lizards (Pettigrew and Bull 2011) or result in the decrease of food resources (Woodcock, Pywell et al. 2005, Pafilis, Anastasiou et al. 2013). All these negative effects would potentially weaken the physiological condition of lizards which could in turn enhance their probability of being infested by parasites, and ultimately lead to the decline of population density (Pafilis, Anastasiou et al. 2013). Research from Pafilis, Anastasiou et al. (2013) corroborated our conclusion, that lizards in grazing areas have a higher tick infestation rate. The existence of large animals in the habitat is a prerequisite for the survival of adult female ticks and the maintenance of a tick population (Gray, Dautel et al. 2009), so another more direct effect of grazing on host-parasite relationship is that many livestock carry ticks

which could parasitize lizards (Liebisch 1997, Casher, Lane et al. 2002), therefore enhancing the prevalence of ticks in grazing areas. Moreover, increased human mediated disturbance (e.g. livestock, human activity, tourism) will also affect the body condition of lizard and the host-parasite relationship by stimulating the antipredatory behaviors of animals (Frid and Dill 2002, Amo, López et al. 2006). Interestingly, Amo, López et al. (2006) proved that wall lizards (*Podarcis muralis*) inhabited in areas with higher levels of tourism had a more serious infestation by ticks. We believe that the livestock activity and human disturbance in our case will also cause the decrease of foraging efficiency (Pérez-Cembranos, Pérez-Mellado et al. 2013), reproduction (Martín, P.López et al. 2003) and body condition (Pérez-Tris, Díaz et al. 2004, Garrido and Pérez-Mellado 2015), which will in turn depress the immune system reactivity and finally increase the risk of parasite infection (Smallridge and Bull 2000). As for the explanation of mite presence decreased with human and grazing activity, there is evidence suggesting that disturbances, like livestock grazing, could reduce the quality of soil and successively decrease mite abundance or even mite assemblages (Battigelli, McIntyre et al. 2003, Kinnear and Tongway 2004), this may explain why we found a negative relationship between mite presence and grazing as well as human activity. At last, our results pointed out temperature had negative effects on tick presence. It has long been known that ticks have a restricted preferred thermal range and show avoiding action under higher temperatures (MacLeod 1935, Lees 1948). Furthermore, unfed ticks would express a shorter survival time in desiccating or warmer conditions (Chilton and Bull 1993) and tick activity also reduces when being exposed to high temperature (Gray, Dautel et al. 2009). So together with the reducing tick survival and activity, common lizard in warmer populations should take advantage of a lower tick infestation.

Why no competition between mites and ticks?

From our results, we can't conclude whether there is any kind of competition because of the slight negative connection between mites and ticks. To our knowledge, only Schall, Prendeville et al. (2000) tried to explore the interaction between mites and ticks in lizards. According to their results, the patterns of ticks and mites prevalence were far different which may suggest that these two kinds of parasites have different ecologies. In our case, we found that mites and ticks are associated to different patterns of environmental factors. Mite presence is associated with higher habitat quality and lower anthropogenic effects, whereas ticks presence is conversely affected by these factors, and this could confirm mites and ticks have different ecologies. Therefore, we can argue that diverse selection on external habitat or

resources probably lead to different parasite distribution and population dynamics, which would decrease the chance of competition. Moreover, the weak number of co-infestation in our sample confirms the potential absence of competition (3.99% of 677 lizards). However, even if we focus on the 31 lizards that have been infested by both ectoparasites, we realize that the different attachment sites on the host between ticks and mites may also decrease the possibility of interspecific competition. Previous researches suggested that ticks mainly attach on neck and around forelimb of lizards, where the body regions with high vascularization and thinner skin can be found (Andrews and Petney 1981, Bauwens, Strijbosch et al. 1983). Moreover, attachments under forelimbs could prevent ticks from being removed by the lizards or from being rubbed off by the vegetation (Bauwens, Strijbosch et al. 1983). On the opposite, mites mainly attach on the ventral scales of lizards (Heredia, Vicente et al. 2014). The distinct spatial distributions of mites and ticks on host are most likely due to their different preferences on microhabitats (Choe and Kim 1988). Though our results confirmed that mites and ticks were rarely in direct competition, we can't reject the possibility that the different attachment sites were not due to the ecological niches segregation in the past for the sake of avoiding interspecific competition (Holmes 1973), nor deny that the specific attachment sites are the by-products developed from adaption of previous hosts (Price 1980), i.e. before colonizing the common lizard. Finally, the different exploitation time on lizard also contributes to the inexistence of interaction between mites and ticks (Schall, Prendeville et al. 2000). The duration of attachment on host by mites and ticks might be different (the amount of mites can vary in a single day, Clobert, personal comments) and the difference of engorgement time (Goldberg and Bursey 1991) could also reduce the competition between mites and ticks.

Parasite load and individual phenotype

From our results, males were getting infested by mites more easily than females as well as tended to harbor a greater number of mites. Mites live freely in soil, climbing on the host for engorging blood (Cooper and Jackson 1981). Because male lizard has a greater home range and a higher mobility during the mating season, it is likely that there is a better chance for male lizards getting in touch with mites, which may explain the different vulnerability to parasites between sexes (Bauwens, Strijbosch et al. 1983, Salvador, Veiga et al. 1996). Moreover, increased testosterone may result in costs which reduce male fitness (Cox and John-Alder 2007). High levels of testosterone in males may benefit individual by potentially increasing mating success, however, it will also cause immunological cost (Oppliger, Giorgi

et al. 2004). Indeed, the immunocompetence handicap hypothesis (Folstad and Karter 1992) postulates that testosterone enhances the expression of male secondary sexual traits with the cost of suppressing immune function, hence triggering hosts facing a higher risk of parasite infestation (Roberts, Buchanan et al. 2004). Similar results obtained from other lizard system also indicated that higher testosterone level would result in a higher parasite infestation in male individuals (Cox and John-Alder 2007).

Irrespectively from their sex, lizards with longer snout-vent length and lower body weight were also more susceptible to mites. Usually, we consider lizards with lower weight/SVL ratio as in a poor body condition (see Oppliger and Clobert 1997 for the index of corpulence) and may fail to express a strong immune response (Navarro, Marzal et al. 2003), and this will lead to host being exposed in a higher probability of being contagious by parasites. The relationship with size is however more difficult to understand since lizard size and survival are curvilinearly related (Ronce and Olivieri 1997). It might be that individuals with long SVL are old (and hence are senescing) or that size and age going along (Duellman and Trueb 1986) that had more time to accumulate mites. Unfortunately, we had no data on the survival of these individuals which might have help us discriminating among these hypotheses.

However, neither tick presence nor abundance was correlated with lizard individual phenotype. It is widely known that males have higher mobility and larger home range (Bauwens, Strijbosch et al. 1983, Salvador, Veiga et al. 1996) so that they will contact with parasite more, but we should also notice that the tick which we study may be not the natural type tick in the field. Furthermore, the distribution of tick is not random because the maintenance of a tick population is dependent on large animals (Gray, Dautel et al. 2009), and in our population tick presence is associated with grazing activity (Table 2). So the probability of individual being infested by ticks should be more dependent on tick density or distribution rather than the difference of phenotype between individuals. Second, some previous researches suggested that tick had no diminishing effects on lizard fitness (e.g. Bull and Burzacott 1993), these research corroborate our results. It might be that lizard could balance the cost of being infected by ticks and the advantage of dominating in good habitats (Main and Bull 2000). In a nutshell, since it is not that serious or detrimental for lizards being infected by ticks, we therefore infer that lizard may not need to evolve any phenotypic change in response to tick infestation.

Why little effects on reproductive effort and offspring morphology?

Life history predict that as reproduction and immune defense are both energy costly, individuals must trade off their allocation of resources on these two life history traits (de Jong, Fokkema et al. 2014). Our results indicated that neither mites nor ticks have an effect on clutch size. In the same way, former researches on mites and blood parasites on common lizard didn't detect any impacts on litter size (Sorci, Massot et al. 1994, Sorci, Clobert et al. 1996). Parasites were however associated to changes in other reproductive parameters. Mite presence is negatively associated with relative clutch mass (RCM) but positively with hatchling success. One explanation of this result is that under a parasite attack, hosts allocate more resources and energy to the immune defense rather than to the reproductive effort, i.e. parasitism affected the decision of host on allocating limited resources (Lehmann 1993, Møller, Clayton et al. 1997). We indeed know that mite load is correlated with increased host mortality (Sorci and Clobert 1995) and we also observed that some lizards die directly because of being heavily infested by mites during rearing (Sorci, Massot et al. 1994).

More surprisingly, mite presence was correlated with a higher survival at lizard hatching. This result goes against previous researches which indicated that ectoparasites reduce reproductive success and increase cost of reproduction (Møller 1993, Oppliger, Richner et al. 1994). However, we should notice that parasitism is not the only factor which could influence host reproductive success. For example, reproductive success declines with timing of breeding and increases with previous successful experience, also inferred age have important influence on reproductive success (Lewis, Wanless et al. 2006). So we can't conclude that mite infestation is reason for increased host hatchling success in our case. It might be that parasite load and female age are linked, hence a relationship with hatching success also increase with female age.

Not such relationship was found with ticks. We only found that ticks correlated with an earlier reproduction of their host. Parasite population and host infestation risk are known to increase with breeding process (Merino, Møller et al. 2000), and host immune competence will decrease when they approach laying date (Sorci, Soler et al. 1997). So it might be that when female are approaching laying date, they are more susceptible to parasites, hence a correlation between laying date and parasite could have been detected.

Mites and ticks have no effects on offspring morphology in our study. Offspring size is affected by many parameters (Uller and Olsson 2010). Except parasitism, factors like laying date, clutch size, food supply and environment etc. all have strong influences on offspring size (Olsson and Shine 1997, Abell 1999). So it is possible that parasite have only a secondary

effect on offspring size or in very particular situations, not represented in our sample. On the other hand, only focusing on offspring traits like body size might lead to erroneous conclusions on parasite mediated offspring future live (Uller and Olsson 2010). Failure to detect parasite-induced morphology variation doesn't mean that parasites have no effects on offspring at all. For example, a previous research on the common lizard failed to detect any effects of mites on offspring morphology (Sorci, Massot et al. 1994), but revealed that offspring from infested mothers had higher values of some fitness components in early life. Thus, our results should be taken cautiously since no information on the long term effects was available for our populations.

In conclusion, we demonstrated that mite and tick were mediated by different patterns of environmental factors, and the different ecologies which may explain why there is no clear co-occurrence between mite and tick. Mite infested mother invested less in reproduction but possessed a high hatching success, whereas tick infested mother reproduced earlier. Neither mite nor tick had any effects on offspring morphology. Thus our research testifies the different ecologies of mites and ticks and indicates the importance of specifying different parasites in future host-parasite research.

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CHAPITRE A3: Plasticité, taille de niche et aire de répartition des principales essences forestières européennes.

Ce chapitre est le fruit de mon travail de Master 2, mené avec Anne Duputié, Isabelle Chuine et Ophélie Ronce, au Centre d'Ecologie Fonctionnelle & Evolutive de Montpellier. S'il ne concerne pas directement le travail de thèse, la problématique reste très liée. De plus, les dernières analyses et la rédaction du manuscrit ont été effectuées au cours de ma première année de doctorat.

Phenological plasticity will not help all species adapt to climate change

ANNE DUPUTIÉ^{1,2*}, ALEXIS RUTSCHMANN^{2,3*}, OPHÉLIE RONCE⁴ and ISABELLE CHUINE²

¹Laboratoire EEP, CNRS UMR 8198, Université Lille1, F-59655 Villeneuve d'Ascq Cedex, France, ²CEFE UMR 5175, CNRS, Université de Montpellier, Université Paul-Valéry Montpellier, EPHE, 1919 route de Mende, 34293 Montpellier Cedex 05, France,

³Station d'Ecologie Expérimentale du CNRS à Moulis, Unité de Service et Recherche 2936, 09200 Moulis, France, ⁴Institut des Sciences de l'Evolution Université Montpellier 2, CNRS, IRD, CC65, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France

Abstract

Concerns are rising about the capacity of species to adapt quickly enough to climate change. In long-lived organisms such as trees, genetic adaptation is slow, and how much phenotypic plasticity can help them cope with climate change remains largely unknown. Here, we assess whether, where and when phenological plasticity is and will be adaptive in three major European tree species. We use a process-based species distribution model, parameterized with extensive ecological data, and manipulate plasticity to suppress phenological variations due to interannual, geographical and trend climate variability, under current and projected climatic conditions. We show that phenological plasticity is not always adaptive and mostly affects fitness at the margins of the species' distribution and climatic niche. Under current climatic conditions, phenological plasticity constrains the northern range limit of oak and beech and the southern range limit of pine. Under future climatic conditions, phenological plasticity becomes strongly adaptive towards the trailing edges of beech and oak, but severely constrains the range and niche of pine. Our results call for caution when interpreting geographical variation in trait means as adaptive, and strongly point towards species distribution models explicitly taking phenotypic plasticity into account when forecasting species distribution under climate change scenarios.

Keywords: climate change, climatic niche, European beech, phenology, Scots pine, sessile oak, species distribution model

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Introduction

Concerns are rising about the capacity of species to adapt quickly enough to global warming (Burrows *et al.*, 2011; Dawson *et al.*, 2011; Hoffmann & Sgrò, 2011). These adaptations imply genetic changes as well as nongenetic changes in trait values (Hoffmann & Sgrò, 2011; Merilä & Hendry, 2014). In long-lived organisms such as trees, genetic adaptation is slow (Savolainen *et al.*, 2004), and how much phenotypic plasticity, that is the production of several phenotypes from a single genotype in different environmental conditions, can help them cope with climate change remains largely unknown (Anderson *et al.*, 2012; Franks *et al.*, 2014; Merilä & Hendry, 2014).

Phenotypic plasticity is adaptive when the phenotype changes in a direction favoured by selection in the new environment (Conover & Schultz, 1995); that is, the phenotypic change results in higher fitness than if there was no phenotypic change. For instance, Great Tits adjust their laying date according to spring tempera-

ture, which allows matching the timing of high food demand with peaks of insect abundance, thus mitigating the impact of climate change (Charmantier *et al.*, 2008). Phenotypic plasticity can, however, result in imperfect adaptation in a changing climate, requiring further genetic changes to reduce maladaptation (Gienapp *et al.*, 2013).

As phenotypic plasticity determines fitness in spatially heterogeneous or changing environmental conditions, it is necessarily related to range size and climatic niche breadth, that is, respectively, the geographical and climatic spaces where fitness is not null. In the context of climate change, adaptive phenotypic plasticity is thus expected to mitigate fitness losses, resulting in broader range and climatic niche than in the absence of plasticity. Hence, adaptive reaction norms would result in fewer extinction rates at the trailing edge of the range and/or in wider colonizable areas at the leading edge. Theoretical models have indeed shown that adaptive phenotypic plasticity can limit range contraction under a changing climate (e.g. Valladares *et al.*, 2014) and help further genetic adaptation to stressful environment by slowing down the population decline in those environments (Chevin *et al.*, 2010). However, phenotypic plasticity can also be maladaptive (Ghalambor *et al.*, 2007),

Correspondence: Anne Duputié, tel. +33 3 20 43 49 91, fax +33 3 20 43 69 79, e-mail: anne.duputie@ens-lyon.org

*Equal contribution.

in particular when environments become unpredictable, thus yielding inappropriate responses and potentially important demographic costs (Reed *et al.*, 2010). Both adaptive and nonadaptive plasticity will play a role in the responses of biodiversity to climate change (Nicotra *et al.*, 2010). Distinguishing between the two thus represents an important research challenge.

Because trees are long lived, they experience variable environmental conditions throughout their lifetime and are likely to show high levels of plasticity. Because of their long generation time, trees are also likely to cope with environmental changes more rapidly through plastic responses than through microevolution (Savolainen *et al.*, 2004; Chevin *et al.*, 2013; Franks *et al.*, 2014). Phenological traits, that is the timing of occurrence of phases of the life cycle, are highly plastic. They vary among places and years, depending mostly on temperature (Schwartz, 2003). The reaction norms of phenology to temperature in plant species result from natural selection, optimizing growing period and reproduction under given environmental conditions. This optimization has to cope with several trade-offs. For example, in boreal and temperate regions, there is a trade-off between maximizing annual carbon assimilation – which favours early leaf unfolding – and reducing the risk of damage caused by frost on vegetative organs – which favours late leaf unfolding (Chaine, 2010). In addition, in ectotherms such as plants, temperature also affects phenology directly by influencing the rates of biochemical processes, which complicates the interpretation of phenotypic plasticity, as it may reflect both physiological constraints and adaptive strategies. Inadequacy of phenology to local climates, leading to failure of mature seed production or bud dormancy release, often explains the position of the northern and southern range limits of trees (Morin *et al.*, 2007). More generally, plant phenology is a major component of fitness and contributes to shape plant species' ecological niches and geographical distributions (reviewed in Chaine, 2010).

By far, most observations of climate change responses have involved alterations of species' phenology (Parmesan, 2006). Reported changes in phenology are mainly advanced spring events, but also delayed fall events. A large part of these shifts in phenology is due to phenotypic plasticity (Franks *et al.*, 2014). Whether these plastic shifts in phenology are adaptive in the context of climate change is, however, open to debate (Chaine, 2010). For instance, earlier growing season in warmer climate could expose populations to drought stress in Mediterranean regions (Misson *et al.*, 2011). In a recent study, Amano *et al.* (2014) found that species showing the weakest changes in flowering dates showed the largest extinction rates at the trailing edge and/or fastest poleward shifts of their range. The

contribution of phenological plasticity to fitness is not easy to measure empirically, especially for long-lived species such as trees; the spatial and temporal scales at which it is adaptive is still unclear. These empirical difficulties are even more acute when trying to predict the adaptive value of current phenological plasticity in future climatic conditions.

Here, we assess whether and where the plasticity of phenological traits is adaptive in three common European trees (European beech *Fagus sylvatica* L., sessile oak *Quercus petraea* (Matt.) Liebl. and Scots pine *Pinus sylvestris* L.) using a process-based species distribution model that describes precisely the phenology of the entire annual cycle. The process-based submodels that describe the reaction norms of each species' phenology to temperature are parameterized using phenological observations in natural populations across the species distribution. These reaction norms generate both interannual and geographical variations of phenological traits and long-term trends under warming climatic conditions (e.g. earlier spring phenology; Parmesan, 2006). For each species, we generated virtual populations with altered levels of plasticity, suppressing the expression of either (i) interannual variation alone, or (ii) both interannual and spatial variations, or (iii) interannual, spatial and trend variations in phenological traits in response to temperature variation. We compared these virtual populations to normally plastic populations to assess how interannual, spatial and trend variation in phenology due to plasticity impacted fitness, and therefore niche breadth and geographical range, in current and future climatic conditions.

Materials and methods

The process-based species distribution model PHENOFIT

PHENOFIT (Chaine & Beaubien, 2001) is a process-based species distribution model developed for temperate trees. It assumes that the reproductive output and survival of an average individual depend on the dates of occurrence of key phenological events (leaf unfolding, flowering, fruit maturation and leaf senescence) and on its ability to resist temperature and water stresses. Figure 1 gives insight into the submodels determining survival and reproductive output from phenology and abiotic stresses, depending on daily temperature, precipitation and photoperiod.

Each phenological model implemented in PHENOFIT assumes a different reaction norm of tissues to temperature. In this study, leaf unfolding and flowering dates are determined using a sequential model that describes an endodormancy phase (bud development is stopped even in optimal meteorological conditions) and a subsequent ecodormancy phase (bud development is stopped only in adverse meteorological conditions). While the endodormancy requires exposure to

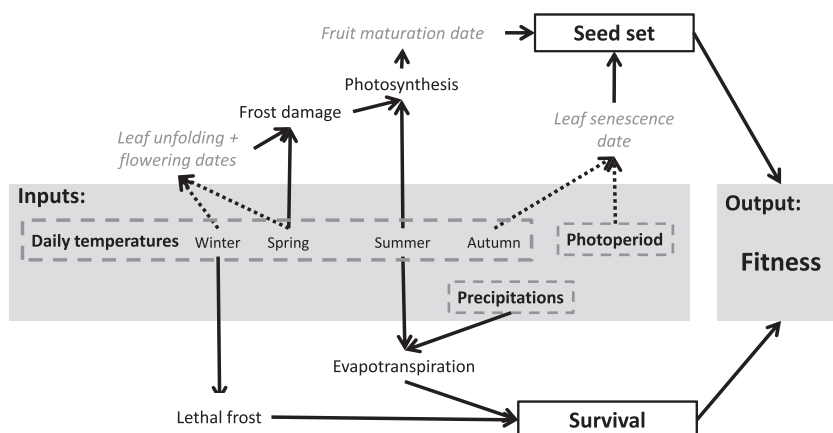


Fig. 1 The model PHENOFT determines the fitness of an average individual (as the product of seed set and survival) as a function of environmental variables (daily temperatures, photoperiod and precipitation). Arrows show the dependency of each process to environmental drivers or phenological events (grey italics). Each arrow thus represents a reaction norm, calibrated either on time series observations or on experiments. Dotted arrows show the reaction norms that were suppressed in the virtual species with altered levels of plasticity.

cool temperatures for several days to be broken (i.e. chilling), the ecodormancy phase requires warmer temperatures to finish. During each phase (endodormancy and ecodormancy), each species shows a specific reaction norm to temperature. The date of fruit maturation depends on photosynthetic ability, which itself depends on temperature, available water in the soil and the proportion of leaves not destroyed by frost. Leaf senescence is modelled as a function of photoperiod and temperature following the study of Delpierre *et al.* (2009). For each event and each species, reaction norms to temperature, photoperiod and water availability are inferred statistically (see below) using time series data from different sites and different years, that is under a wide range of climatic conditions. These reaction norms are responsible for the temporal and geographical variations of the dates of leaf unfolding, flowering, fruit maturation and leaf senescence (deciduous) or bud set (evergreen) observed in natural populations. In this study, we used the local 20-year average of the product of survival and reproductive output as a proxy for fitness (ranging from 0 to 1). Details on the model are available in the online Supplementary Information file (Section S1).

Climatic data

For each species, the model PHENOFT was used to compute the yearly phenology, reproductive success and survival of the species over Europe (11°W, 34°N to 32°E, 72°N) over 1981–2000 and 2081–2100 following two climatic scenarios, A1Fi ('business-as-usual') and B2 (lower greenhouse gas emissions; IPCC, 2000). The model was driven by daily climatic data obtained from the general circulation model HadCM3 (Hadley Centre Coupled Model 3, MetOffice Hadley Centre for Climate Change, Exeter, UK, Gordon *et al.*, 2000) downscaled by the ATEAM project at 10' resolution (Mitchell *et al.*, 2004). These data are monthly mean values corrected by their anomalies on the benchmark period (1961–1990, 30 years of mean

climate data). Because daily climate data are needed to run PHENOFT, we used a stochastic weather generator to create daily temperatures (Nicks *et al.*, 1995; Morin & Chuine, 2005).

Parameterization of observed and altered phenological plasticity

Using a simulated annealing algorithm, we parameterized the different phenological models using time series observations of the different phenological events in natural populations and corresponding daily temperatures, for three common European tree species: European beech *Fagus sylvatica*, sessile oak *Quercus petraea* and Scots pine *Pinus sylvestris*. Although reaction norms of phenology to climatic cues may vary across the range of a species, we deliberately fitted a single phenological model per event (leaf unfolding, flowering, fruit maturation and leaf senescence) and per species for two reasons. First, our objective here was to disentangle the effects of plasticity on fitness and to decompose the interannual, spatial and trend effects of the reaction norms. We here assess the fitness effect of the spatial variation in phenological traits due to spatial variation in climates only, excluding variation due to potential local adaptation of the reaction norms of phenology to climate. Second, accounting for local adaptation throughout species' ranges requires long-term accurate phenological records from many populations scattered across the range. Data currently available do not meet this requirement.

We assessed the validity of model projections (without altering plasticity) over the historical time period by comparing modelled fitness to the known distribution of the species, using consensual observed distribution maps (see Supplementary Information Section S2; Duputié *et al.*, 2014). The discriminatory power of the model was fair to good, with areas under the receiver operating curves (Swets, 1988) varying from 0.645 to 0.875 depending on the species (Section S2). This shows that the model can produce a fair representation of observed distri-

butions, even when local adaptation in phenological reaction norms is not taken into account.

Phenological observations of natural populations were obtained from the French and European Phenological Databases (Observatoire des Saisons, <http://www.gdr2968.cnrs.fr/> and PEP 725, <http://www.pep725.eu/>). For *Fagus sylvatica*, leaf unfolding, leaf senescence and flowering date observations were obtained at the European scale (models were calibrated with 575, 560 and 23 observations, respectively), but fruit maturation observations were only collected from French sites (23 observations). For *Quercus petraea*, all event dates were obtained from French sites (522 observations of leaf unfolding, 202 for flowering, 296 for fruit maturation and 228 for leaf senescence). For *Pinus sylvestris*, we did not calibrate phenological models, but used those prescribed by Kramer (1994), which were obtained on a German provenance. Detailed information on the data used is provided in the Supplementary Information file (Section S1).

For each species, PHENOFIT was run using the same climatic data sets but with four nested degrees of phenological plasticity (hereafter called 'Models') (Table 1). In model 0, plasticity was not altered: the empirically determined reaction norms determined the date of occurrence of each phenological event, each year and at each location. In models 1, interannual variation in phenological dates was suppressed. Event dates were imposed at their local 20-year average, and fitness was computed given that constraint. In models 2, phenological dates were set to their 20-year rangewide average according to model 0 for the period considered. Models 2 thus additionally remove geographical variation in phenology due to plasticity. Models 0, 1, 2 were run under historical and future climatic conditions. Models 3 were run under future climatic conditions only. In these models, phenological dates were held at their 1981–2000 rangewide mean, while PHENOFIT was run with future climatic conditions. Models 3 thus further removed the trend variation in phenological traits, due to the expression of phenological plasticity under warmer climates. Our treatments thus progressively remove the spatiotemporal variation of spring and fall event dates due to phenotypic plasticity,

around their mean values. Different predictions about the adaptive value of plasticity would be obtained if different values were used as a nonplastic reference (e.g. earliest or latest recorded date for an event). Exploring such alternative contrasts could inform us on the adaptive value of delaying or accelerating phenology in different locations and years but is out of the scope of this study.

Note that models 0–3 are nested. Comparing models 0 and 3 (or 2 for historical conditions) provides the total contribution of phenological plasticity to fitness. Comparing models 0 and 1 provides the contribution of interannual variation in phenology to fitness. Comparing models 2 and 1 provides the additional contribution of spatial variation in plasticity to fitness. Comparing models 3 and 2 provides the additional contribution of the trend variation in phenology due to global warming (Table 1). Here, phenological plasticity will be considered adaptive when and where fitness is higher in a plastic treatment than in a nonplastic one (e.g. when fitness is higher in model 0 than in models 1, 2 or 3).

The four models were applied to the following traits: (i) spring events (i.e. leaf unfolding and flowering dates – models 1a, 2a and 3a), (ii) fall events (i.e. leaf senescence date – models 1b, 2b and 3b) and (iii) spring and fall events dates (models 1c, 2c and 3c; Table 1). Note that only spring events were manipulated in the evergreen *Pinus sylvestris*. Dates of leaf unfolding and flowering were always set jointly because they are determined largely by the same cues and are highly correlated. The plasticity of the fruit maturation date, which is tightly linked to reproductive success, was not altered for any species, because removing variation in this trait amounted to removing most variation in fitness. Dotted arrows on Fig. 1 show the relationships that were bypassed in PHENOFIT to create the altered plasticity models.

Analysis of simulations

Fitness distribution maps of models were compared on a pixel-by-pixel basis in either the geographical or the climatic space (see Fig. S2 for a worked example). To describe the cli-

Table 1 Nested model design showing which sources of phenological variation are included in the models (X) or not (–). Comparing models 0 and 1 provides the fitness contribution of interannual variation in phenology due to plasticity; comparing models 1 and 2 provides the further fitness contribution of spatial variation in phenology due to plasticity; and comparing models 2 and 3 provides the additional contribution of the trend variation in phenology under warmer climates

Source of variation	Interannual	Spatial	Trend
Model 0: Reference model with empirically fitted reaction norms Dates of spring and fall events vary with locality and across years.	X	X	X
Models 1: Removing the effect of year-to-year fluctuations Dates of spring (1a), fall (1b), or spring & fall events (1c) are forced to their <i>local period average</i> .	–	X	X
Models 2: Removing the effect of spatial variation Dates of spring (2a), fall (2b), or spring & fall events (2c) are forced to their <i>rangewide period average</i> .	–	–	X
Models 3: Removing the effect of trend variation Dates of spring (3a), fall (3b), or spring & fall events (3c) are forced to their <i>rangewide 1981–2000 average</i> .	–	–	–

matic space, we used the first two axes of a principal component analysis conducted on the concatenated climatic data sets (historical and scenarios) of Europe, using eight bioclimatic variables (as in Gritti *et al.*, 2013): mean temperature of the coldest month (°C), mean temperature of the warmest month (°C), number of chilling days, a drought index (Sykes *et al.*, 1996), growing day degrees above 5 °C (°C. day), total amount of precipitation (mm), amount of precipitation during the growing season (mm) and the coefficient of variation of precipitations among seasons. The first axis is mostly driven by temperature variables and the second axis by precipitation variables; they together explain 81% of the total variance.

To compute modelled range area and niche breadth, we considered pixels of the geographical (or climatic) space with mean fitness exceeding 0.1 over the considered period as potentially harbouring the species (or virtual species with altered levels of plasticity). Using this threshold resulted in realistic representations of the species' observed distributions (Section SII.2).

Under climatic scenarios, we distinguished five types of pixels: newly colonized, increase in fitness, decrease in fitness, gone extinct or species absent under current and future conditions. To establish whether plasticity differently affected fitness in these five categories of pixels, we generated generalized least squares models explaining fitness differences among models as a function of pixel type, and assuming spatial autocorrelation followed a Gaussian kernel. Due to computational constraints, these models were established on a subsample of the 10' grid, resampled at a 0.5° resolution, containing 3740 points. Significance of pairwise *t*-tests was assessed using the multcomp R library. All analyses were performed in R 3.0.2 (<http://www.r-project.org/>).

Results

Is phenological plasticity adaptive under historical climatic conditions?

Comparing models 2c and 0 under historical conditions (1981–2000) gives access to the total contribution of phenological plasticity in all traits to fitness under current conditions. Plasticity increases the niche breadth of sessile oak (4.2%) and beech (10.8%) and the range size of beech (6.7%), while it decreases that of oak (−1.5%; Table 2). Note that because geographical locations are not evenly spread in the climatic space (Fig. S3), an increase in climatic niche breadth does not always translate into an increase in range size. In contrast, phenological plasticity strongly decreases the niche breadth (−22.3%) and range size (−15.9%) of Scots pines (Table 2).

Plasticity in phenological events is mostly neutral throughout the niche and distribution of all three species (Fig. 2). For beech and oak, phenological plasticity is strongly adaptive towards the warm (low latitude or altitude) margins, but maladaptive towards their cold

margins (high latitude or altitude). By contrast, in Scots pine, phenological plasticity is rarely adaptive and is maladaptive towards both the warm and – to a lesser extent – cold margins of its niche and distribution in Western Europe (Fig. 2).

The effects of plasticity of spring (Fig. S4) or fall event dates only (Fig. S5) on niche breadth and range size are qualitatively the same (Table 2).

Is phenological plasticity adaptive under future climatic conditions?

Future European climatic space and climatic niches of all three species are projected to expand (Figs 2 and S3), under both scenarios (+18.5–41.9% by 2081–2100 for the climatic niches). Indeed, new combinations of climatic variables are projected to appear, some of which are favourable to these species (especially with moister climates). However, these favourable conditions are projected to appear in scarce locations (Fig. S3); hence, the potential distribution of all three species is projected to shrink by 2.8–17.3% under scenario A1Fi. Under the less extreme scenario B2, the geographical range of Scots pines is projected to shrink by 3.5%, while those of beech and oak is projected to expand by 3.4–6.8% (Fig. S6).

Under scenario A1Fi, plasticity strongly increases niche breadth (by 35.3% and 17.8%) and range size (by 55.1% and 39.7%) in beech and oak, respectively (Table 2). Positive effects of plasticity are widespread through the niches and ranges of beech and oak, especially towards the southern part of their future range – which may include newly colonized regions (Table 2 and Figs 2–3, bottom rows). In Scots pine, the negative effect of plasticity on fitness observed under historical climates increases under scenario A1Fi and results in a 32.8% loss of niche breadth and a 45.0% loss of geographical range (Table 2 and Figs. 2–3, bottom rows). Milder but consistent effects are observed for all three species under scenario B2 (Table S3, Figs S7 and S8).

How do interannual, spatial and trend variation in phenology impact fitness?

The nested design of the models allows us to disentangle how interannual (model 1 – model 0), spatial (model 2 – model 1) and trend variation in traits (model 3 – model 2) affect fitness. Interannual variation in phenology due to plasticity in spring and fall events considered jointly has a surprisingly low, yet positive, effect on niche breadth and range size (0.5–16.8%; Table 2). However, in beech and oak, interannual variation in fall event dates negatively affects fitness (Table 2). In Scots pine (and to a lesser extent in oak),

Table 2 Changes in climatic niche size and geographical range size due to various components of phenotypic plasticity (see Table 1), under historical climatic conditions and scenario A1Fi. In the geographical space, 10' pixels are weighted by their geographical area. Figures shown are relative to the 'plastic' run (model 0)

			<i>Fagus sylvatica</i> (%)		<i>Quercus petraea</i> (%)			<i>Pinus sylvestris</i> (%)	
			2081–2100		2081–2100			2081–2100	
Trait	Contribution of expressed part of plasticity (relative to model 0)		1981–2000	A1Fi	1981–2000	2081–2100	A1Fi	1981–2000	A1Fi
Niche change									
Spring events (models a)	Interannual	0–1a	1.8	1.8	3.5	3.1		3.0	7.9
	Spatial	1a–2a	1.9	8.3	–1.8	–7.2		–25.3	–39.3
	Trend	2a–3a	–	5.3	–	7.8		–	–1.4
	Total	0–3a or 0–2a	3.7	15.4	1.7	3.6		–22.3	–32.8
Fall events (models b)	Interannual	0–1b	–0.6	–1.6	–0.4	–1.4			
	Spatial	1b–2b	4.8	18.2	4.5	6.7			
	Trend	2b–3b	–	15.3	–	10.6			
	Total	0–3b or 0–2b	4.2	31.9	4.1	15.9			
Both (models c)	Interannual	0–1c	1.1	0.5	2.6	1.5			
	Spatial	1c–2c	9.7	22.3	1.7	3.6			
	Trend	2c–3c	–	12.5	–	12.7			
	Total	0–3c or 0–2c	10.8	35.3	4.2	17.8			
Range change									
Spring events (models a)	Interannual	0–1a	2.0	3.3	7.9	5.5		3.4	16.8
	Spatial	1a–2a	2.3	14.9	–10.1	1.3		–19.3	–59.7
	Trend	2a–3a	–	9.0	–	13.3		–	–2.0
	Total	0–3a or 0–2a	4.3	27.2	–2.2	20.1		–15.9	–45.0
Fall events (models b)	Interannual	0–1b	0.0	–2.2	–0.5	–0.8			
	Spatial	1b–2b	1.8	20.5	–1.5	15.1			
	Trend	2b–3b	–	17.0	–	14.8			
	Total	0–3b or 0–2b	1.7	45.3	–2.0	29.1			
Both (models c)	Interannual	0–1c	2.0	0.6	7.4	4.3			
	Spatial	1c–2c	4.7	27.4	–8.9	17.4			
	Trend	2c–3c	–	27.2	–	18.0			
	Total	0–3c or 0–2c	6.7	55.1	–1.5	39.7			

interannual variation of phenological dates shows curious effects around the margins, with a negative effect within the occupied range at the margin, a neutral effect at the margin and a positive effect outside the margin (Fig. 4).

Further removing spatial variation in phenology has a much larger effect on fitness, range width and niche breadth, explaining a large part of the total effect of removing phenotypic plasticity, with, in consequence, similar patterns of variation in the sign and extent of these effects among species, time periods and positions in the climatic and geographical space, as described for the total effect of plasticity above (Table 2, Fig. 2). This is because climatic variations across the geographical range of the three species are much stronger than those encountered across years in any given location.

The observed reaction norms of phenological events further affect fitness under warmer climates. For beech and oak, phenological plasticity tends to advance spring events and to delay fall events under warmer climates. This trend variation positively affects fitness at

the core and trailing edge of the projected distributions of both species (Fig. 3). For Scots pines, spring events are projected to be delayed in most of its current range under warmer climate; hence, the trend variation in spring events due to phenological plasticity negatively affects fitness towards the trailing edge and most of the distribution of this species (Fig. 3).

Discussion

Determining the contribution of phenotypic plasticity to fitness, niche breadth and range size is not an easy task, especially for long-lived species. In temperate trees, leaf unfolding and flowering phenology are constrained by two opposite selective pressures: early leaf unfolding increases carbon assimilation and early flowering increases the probability to achieve fruit maturation before adverse meteorological conditions arise, but they both also increase the probability of spring frost damage on leaves and flowers or developing fruits. Temperature also drives the rates of biochemical

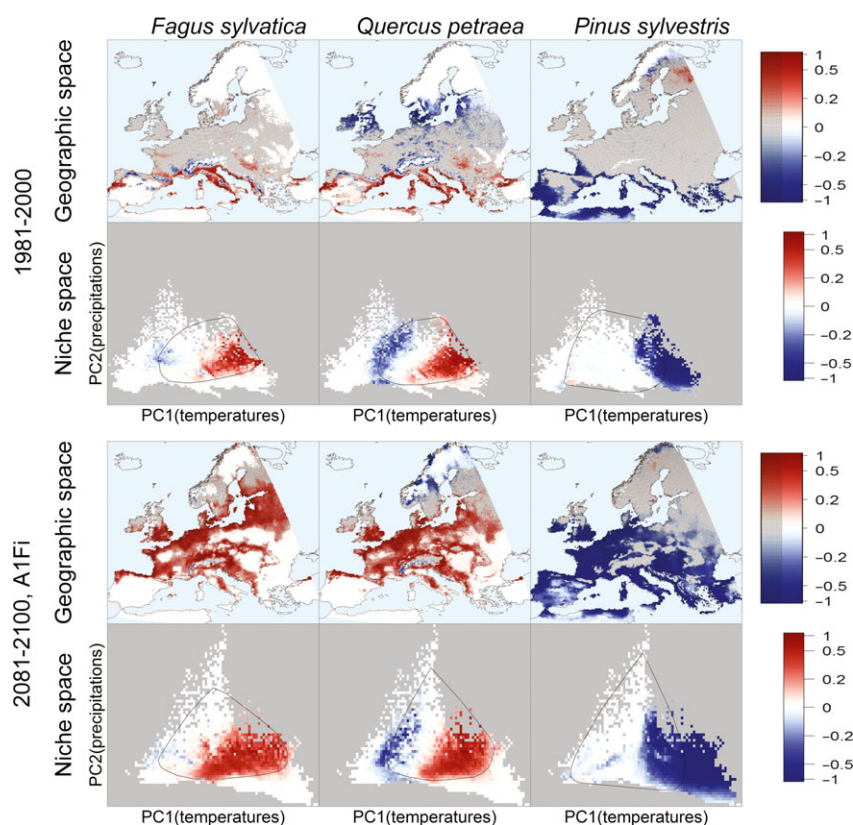


Fig. 2 Total contribution of plasticity to fitness, in the geographical (top and third row) and climatic spaces (second and bottom row), under historical conditions (1981–2000, model 0 – model 2c; top two rows) and under scenario A1Fi (2081–2100; model 0 – model 3c; bottom two rows). Blue zones indicate locations where phenological plasticity is costly and red zones, where it is advantageous. Black dots in the geographical space and black lines in the climatic space represent the regions where modelled fitness in model 0 exceeds 0.1. Geographical space: Lambert azimuthal equal area projection.

processes, setting constraints on the timing of organ development. Hence, a plant's phenology results both from physiological constraints and from adaptive strategies. Thus, it may not be adaptive everywhere and/or each year. Using a process-based tree species distribution model, we show that plasticity of phenological traits increases niche breadth and range size of oak and beech, especially in future climatic conditions. This result is in line with the common expectations that plasticity, when adaptive, increases niche breadth (Richards *et al.*, 2006; Willis *et al.*, 2008) and helps species adapt to climate change (Gienapp *et al.*, 2013; Franks *et al.*, 2014). It is also in line with previous studies showing that high plasticity in various fitness-related traits facilitates range expansions during invasions (Davidson *et al.*, 2011). However, we also show that phenological plasticity decreases niche breadth and range size of Scots pine, illustrating the limits of plasticity due to physiological constraints. The common expectation that plasticity increases niche breadth and helps species adapt to climate change therefore thus holds true for some species only.

Different species, different reaction norms and different effects of plasticity

As compared to the two deciduous species, phenological plasticity in Scots pines shows very different effects. This is because the phenology of Scots pines shows a distinct reaction norm of bud development to temperature.

Bud dormancy break requires higher amounts of chilling in Scots pine than in oak and beech (Hänninen & Pelkonen, 1989; Heide, 1993; Vitasse *et al.*, 2010). This generates different spatial trends of leaf unfolding and flowering across the species range (Fig. S10, second row). Beech and oak show a more or less linear trend from earlier dates at the warmer margins to later dates at the colder margins because, while chilling requirements are always met, cell growth rate is higher under warmer climates. In contrast, Scots pine shows a unimodal trend with earlier dates at the core of the distribution where chilling requirements are met and cell growth rate is high, and later dates towards the warmer margins – where insufficient chilling delays dormancy break – and towards the colder margins –

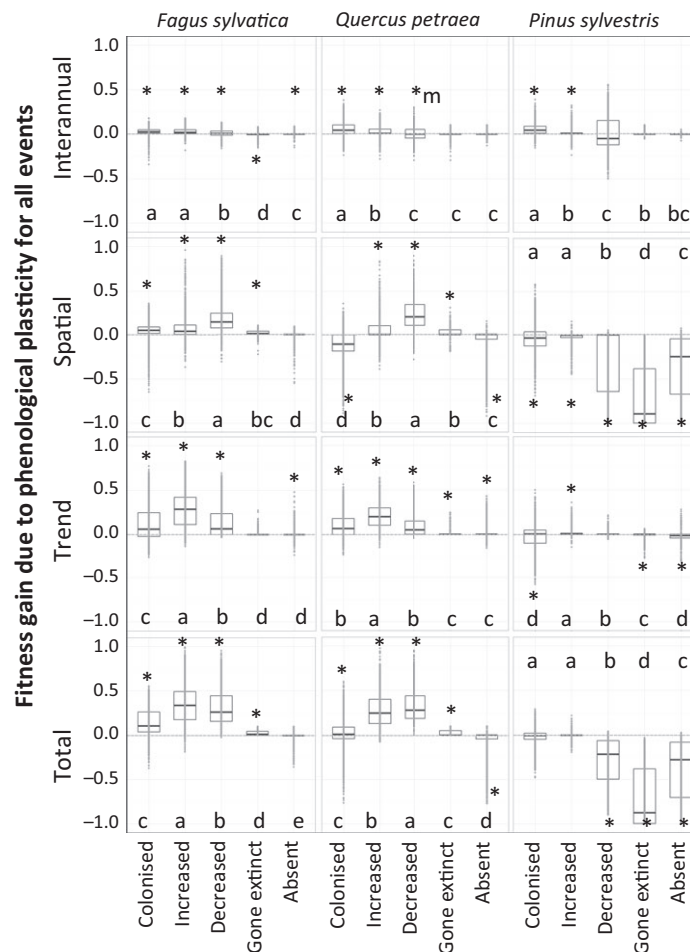


Fig. 3 Box plot showing fitness gains (positive values) or losses (negative values) due to plasticity for the three species (columns), as a function of classes of fitness difference between 2081–2100 (scenario A1Fi) and 1981–2000 (colonized, increased, decreased, gone extinct and absent). Top row: fitness contribution of interannual variation in trait means (model 1c – model 2c). Second row: fitness contribution of geographical variation in trait means (model 2c – model 3c). Third row: fitness contribution of trend variation in trait means (model 2c – model 3c). Fourth row: total contribution of phenological plasticity to fitness (model 0 – model 3c). Stars indicate significant difference from zero at the 5% level, with star location showing the sign of the difference.

where colder temperatures slow down cell growth. The geographical variation in Scots pines' phenology thus also reflects a physiological constraint set by the chilling required to break bud dormancy. Artificially removing the geographical expression of plasticity in Scots pine thus artificially allows the production of fruits in regions where leaf unfolding would naturally occur late in the season, or not at all because of insufficient chilling. Thus, the reaction norm of leaf unfolding date to temperature defines a strong limit to the expansion of Scots pines at the south-western edge of its range.

Among the three species studied here, European beech is the least sensitive to artificial removal of plasticity. Phenology in plants is driven primarily by temperatures and secondarily by photoperiod (Chuine *et al.*, 2013). European beech is known to show a lower

variability in leaf unfolding date than other species, supposedly because this trait is under stronger photoperiodic control in this species compared to others (Vitasse & Basler, 2012). In our simulations, spring event dates indeed showed less variation for this species than for the other two; this may explain why phenological plasticity was found to weakly affect beech fitness.

Why and where is phenological plasticity adaptive?

To understand the different effects of phenological plasticity, one needs to understand the impact of phenology on fitness at the scale of the species' range. Too early leaf unfolding and flowering increase the risk of frost damage, while too late leaf unfolding and flowering compromise the possibility to set fruits.

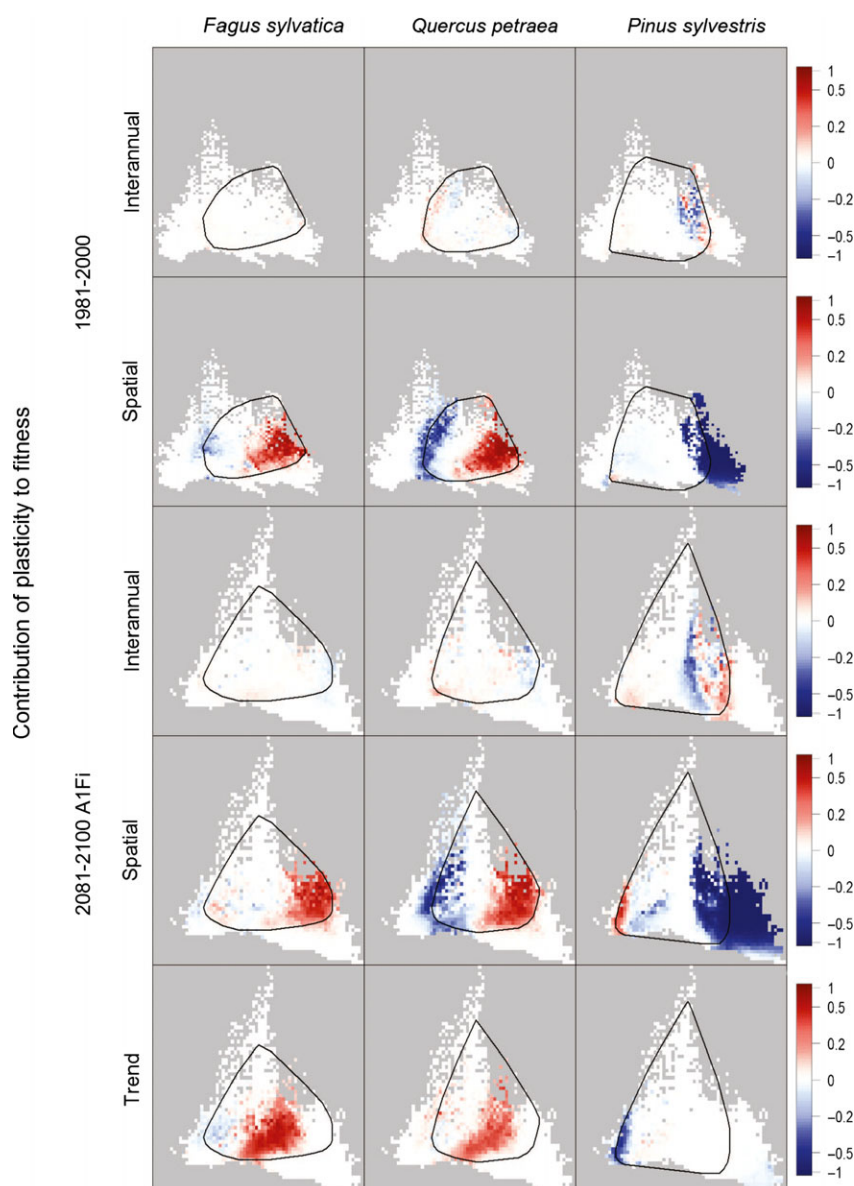


Fig. 4 Fitness gains (or losses) in the climatic space, attributable to the expression of phenological plasticity for all traits (set jointly) among years, across the range and among years under climate warming. The fitness contribution of interannual variation in trait mean is computed as model 0 – model 1c; that of geographical variation in trait mean as model 1c – model 2c; that of trend variation as model 2c – model 3c. Red regions show where plasticity is adaptive; blue regions show where the expression of the trait's reaction norm is maladaptive. The black line indicates the envelope of climatic space where modelled fitness is above 0.1. Note scales differ among rows.

Within the range of all three species, leaf unfolding occurs in a range of 26 (beech), 46 (oak) and 71 (pine) days (Fig. S10) without substantially affecting the ability to set seed (Fig. S11). Thus, within this range, interannual variation in leaf unfolding and flowering dates is almost neutral. Nevertheless, outside this range, fitness decreases rapidly (Fig. S11). Thus, fitness is almost null towards the edges of the species' distributions, where leaf unfolding occurs very late on aver-

age. As a result, interannual variation in phenology can only increase fitness there (see red pixels towards niche margins on the top row of Fig. 4). On the contrary, towards the inner margin of the distribution (and niche), fitness is high on average but not maximal. Hence, interannual variation in phenological dates tends to decrease fitness (blue pixels towards the inner margins of the niche on Fig. 4). Note that at the scale of the whole distribution, the positive fitness

effect of interannual variation in spring events dates dominates over the negative effect of variation in leaf senescence dates (Table 2, and Figs S3 and S4). Indeed, the photosynthesis rate is higher during spring and summer than during fall, and therefore, leaf senescence dates contribute less to fruit maturation than do leaf unfolding dates.

The effect of spatial variation in phenological dates due to plasticity is very contrasted between Scots pine and the two deciduous species because they show different reaction norms to temperature. The spatial variation in phenology due to plasticity negatively affects fitness of the three species towards their colder margins because low temperatures slow down the cell growth which critically delays leaf unfolding and flowering and jeopardize fruit maturation. But its effects at the warmer margins are contrasted: while it positively affects fitness of beech and oak because higher temperatures accelerate the cell growth and result in earlier development, it negatively affects fitness of Scots pine because of the physiological constraint of unmet chilling requirements.

Evolvability of phenological reaction norms

Phenological traits are highly heritable (e.g. Anderson *et al.*, 2012) and tightly linked to fitness (Chuine, 2010; Polgar & Primack, 2011; our results). They are thus expected to respond to selection. Indeed, phenological traits have been shown to have evolved over the last decades in various types of organisms (e.g. Bradshaw & Holzapfel, 2001; Nussey *et al.*, 2005; Kovach *et al.*, 2012; Franks *et al.*, 2014). Local adaptation in phenological traits in trees has also been shown by many provenance trials (Savolainen *et al.*, 2007). For example, the temperature threshold below which winter chill affects dormancy release varies among ecotypes of birch (*Betula* spp, Myking & Heide, 1995). We found that, depending on species, the current reaction norm to temperature produces maladaptive phenology in southern and/or northern margins of the range (or warmer and colder margins of the niche), especially under climate change. We would therefore expect selection to favour a different reaction norm in these marginal localities and selection to intensify as climate warms. However, whether tree populations with a long generation time will be able to evolve fast enough to cope with ongoing climate change remains unclear (Savolainen *et al.*, 2004). How much gene flow from populations where the current reaction norm does not compromise fitness, or enhances it, may constrain the differentiation of locally adapted reaction norms at the margins is also an open question. Further genetic adaptation of the reaction norm to warming climate may

instead be facilitated in localities where plasticity of phenology is adaptive and buys time for adaptation to proceed before extinction (Chevin *et al.*, 2010). In that respect, genetic adaptation is more likely to occur in sessile oak and European beech than in Scots pines: for the latter, plasticity is predicted to be maladaptive towards the trailing edge of the range, where conditions will deteriorate under climate warming, making it even less likely that genetic adaptation can rescue the marginal populations from extinction. In contrast, the exact opposite prediction can be made for oak and beech, with phenotypic plasticity being adaptive precisely in locations threatened by climate warming. Accounting for the differentiation of phenological reaction norms across the range could tamper these predictions (Valladares *et al.*, 2014), especially where phenological plasticity appears to be maladaptive. In this regard, acquiring large-scale phenological data for Scots pines is important, to determine whether the results we obtained for this species are due to phenological reaction norms being calibrated on a single population.

We have shown here that phenological plasticity is not always nor everywhere adaptive and will strongly contribute to either increasing or decreasing species range size under future climatic conditions depending on the species. Our results therefore strongly support the idea that species distribution models should explicitly take phenotypic plasticity into account when trying to forecast the distribution of biodiversity under scenarios of climate change (McMahon *et al.*, 2011; Thuiller *et al.*, 2013; Amano *et al.*, 2014; Valladares *et al.*, 2014). We here illustrate a way to quantify the contribution of phenotypic plasticity to range and niche shifts under climate change in tree species, but similar studies can be conducted for a wider range of organisms, plants and animals, thanks to the growing research on process-based species distribution modelling. Such studies should have a major impact on future research on biodiversity modelling, and on the development of natural resources management and biodiversity conservation strategies.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Section S1. The model PHENOFT and its parameterization.

Table S1. Parameters used in PHENOFT (*Fagus sylvatica* and *Quercus petraea*).

Table S2. Parameters used in PHENOFT (*Pinus sylvestris*).

Section S2. Model validation.

Section S3. Supplementary figures and table

Figure S1: Observed distribution and modelled fitness of the three species with treatment 0, under historical conditions.

Figure S2. Fitness distribution maps of treatments were compared on a pixel-by-pixel basis in the geographical and niche space.

Figure S3. Density of geographical pixels in the climatic space of Europe under historical and future conditions under both scenarios.

Figure S4. As main text Fig. 2 but showing results for manipulation of spring events only.

Figure S5. As main text Fig. 2 but showing results for manipulation of fall events only.

Figure S6. Projected fitness in the 'plastic' treatment for the three species for 1981–2000, and 2081–2100, under the two greenhouse gas emission scenarios A1Fi and B2.

Figure S7. As main text Fig. 2 but showing results for both scenarios.

Figure S8. As main text Fig. 3, but under scenario B2.

Figure S9. As main text Fig. 4 but showing results in the geographical space only.

Figure S10. Leaf unfolding dates in the 'plastic' treatment for the current period, and factors limiting fitness.

Figure S11. Predicted mean seed set of sessile oak (1950–2000) at a location in northeastern France (5°E, 49°N), for various imposed fixed dates of leaf unfolding and flowering.

Table S3. Changes in climatic niche size and geographical range size due to various components of phenotypic plasticity under historical conditions and climatic scenarios A1Fi and B2.

CHAPITRE A4: Impact du climat et de l'habitat sur la phénologie des populations de lézards vivipares.

Version publiée du premier chapitre de cette thèse.

Climate and habitat interact to shape the thermal reaction norms of breeding phenology across lizard populations

Alexis Rutschmann^{1*}, Donald B. Miles^{1,2}, Jean-François Le Galliard^{3,4}, Murielle Richard¹, Sylvain Moulherat^{1,5}, Barry Sinervo⁶ and Jean Clobert¹

¹CNRS, USR2936, Station d'Ecologie Expérimentale du CNRS à Moulis, 09200 Moulis, France; ²Department of Biological Sciences, Ohio University, 131 Life Sciences Building, Athens, OH, USA; ³Laboratoire iEES Paris, CNRS/ENS/UPMC, UMR 7618, Université Pierre et Marie Curie, 7 Quai St. Bernard, 75005 Paris, France; ⁴CNRS/ENS, UMS3194, CEREEP – Ecotron Ile-de-France, Ecole Normale Supérieure, 78 rue du Château, 77140 St-Pierre-lès-Nemours, France; ⁵TerrOïko, 2 rue Clémence Isaure, FR-31250 Revel, France; and ⁶Department of Ecology and Evolutionary Biology, Earth and Marine Sciences, University of California, Building A316, Santa Cruz, CA 95064, USA

Summary

1. Substantial plastic variation in phenology in response to environmental heterogeneity through time in the same population has been uncovered in many species. However, our understanding of differences in reaction norms of phenology among populations from a given species remains limited.

2. As the plasticity of phenological traits is often influenced by local thermal conditions, we expect local temperature to generate variation in the reaction norms between populations.

3. Here, we explored temporal variation in parturition date across 11 populations of the common lizard (*Zootoca vivipara*) from four mountain chains as a function of air temperatures during mid-gestation. We characterized among-population variation to assess how local weather conditions (mean and variance of ambient temperatures during mid-gestation) and habitat openness (an index of anthropogenic disturbance) influence the thermal reaction norms of the parturition date.

4. Our results provide evidence of interactive effects of anthropogenic disturbance and thermal conditions, with earlier parturition dates in warmer years on average especially in closed habitats.

5. Variation in the reaction norms for parturition date was correlated with mean local thermal conditions at a broad geographical scale. However, populations exposed to variable thermal conditions had flatter thermal reaction norms.

6. Assessing whether environmental heterogeneity drives differentiation among reaction norms is crucial to estimate the capacity of different populations to contend with projected climatic and anthropogenic challenges.

Key-words: among-population variation, anthropogenic disturbance, common lizard, parturition date, phenology, plasticity, reaction norm, thermal sensitivity

Introduction

Changes in phenology, the timing of life cycle events, due to climate warming have been well documented during the past decades. A shift in phenology, in particular the onset of breeding, is often considered to be an effective

response by which plants and animals can cope with climate warming (Walther *et al.* 2002). Substantial evidence suggests that phenological traits exhibit plasticity and fluctuate from year to year to track prevailing environmental conditions (Menzel & Fabian 1999; Réale *et al.* 2003; Visser & Both 2005; Charmanier *et al.* 2008). For example, a recent meta-analysis estimated that the current increase in temperatures has advanced the phenology of reproduction and migration in 62% of 678 species

*Correspondence author. E-mail: alexis.rutschmann@eceox-moulis.cnrs.fr

(Parmesan & Yohe 2003). The extent to which a species phenology may respond to climate warming throughout its range depends on spatial variation in the sensitivity of phenology to local climate conditions (Chevin, Lande & Mace 2010; Chown *et al.* 2010). Yet, most shifts in phenology have been observed only within a single natural population. Our current knowledge of temporal variation in phenology across spatially distinct populations is limited to a few long-term studies of breeding dates in avian or mammals populations (Visser *et al.*, 2003; Both *et al.*, 2004; Brommer *et al.*, 2005; Nussey *et al.*, 2005; Husby *et al.*, 2010; Porlier *et al.*, 2012; Sheriff *et al.*, 2011; Visser, te Marvelde & Lof 2012).

An efficient way to explore plasticity in breeding phenology is the study of the ‘reaction norm’ that expresses variation of the breeding dates along an environmental gradient, such as thermal conditions (Brommer *et al.* 2005; Nussey *et al.* 2005; Garant *et al.* 2008). Comparison of the thermal reaction norms for breeding date among populations allows one to estimate the extent of spatial variation in phenotypic plasticity. Studies of multiple populations also provide insights into the causes of variation in thermal reaction norms. Moreover, the understanding of variation in phenology among populations is a unique opportunity to predict how populations should respond to climate change given the anticipated exposure of species to novel combinations of climatic and anthropogenic conditions (Travis 2003; Baumann & Conover 2010; Chown *et al.* 2010).

Several studies have highlighted the potential for strong selection on the timing of breeding phenology (Chaine 2010). First, energetically demanding activities associated with breeding should coincide with periods of suitable food availability, and match the temporal dynamics of prey species in the environment (match–mismatch hypothesis, Sinclair & Tremblay 1984; Miller-Rushing *et al.* 2010; Visser, te Marvelde & Lof 2012). Secondly, phenological traits are often heritable and exhibit significant levels of additive genetic variation, suggesting the potential for an evolutionary response to selection and the local adaptation of breeding phenology traits (Scheiner 2002; Nussey *et al.* 2005; Bradshaw, Holzapfel & Crowder 2006). Different evolutionary and ecological scenarios make different predictions about the degree of variation in breeding phenology reaction norms across populations (Fig. 1). First, breeding phenology may vary across populations even if they share the same reaction norm because populations have different positions on the environmental gradient (Fig. 1a). Secondly, breeding phenology may not differ across populations despite environmental variation because selection promotes physiological or behavioural adaptations such that individuals breed on average at the same dates (Fig. 1b). Finally, populations may evolve a locally adapted reaction norm, influenced by prevailing local conditions, and thus display a locally adapted response (Fig. 1c).

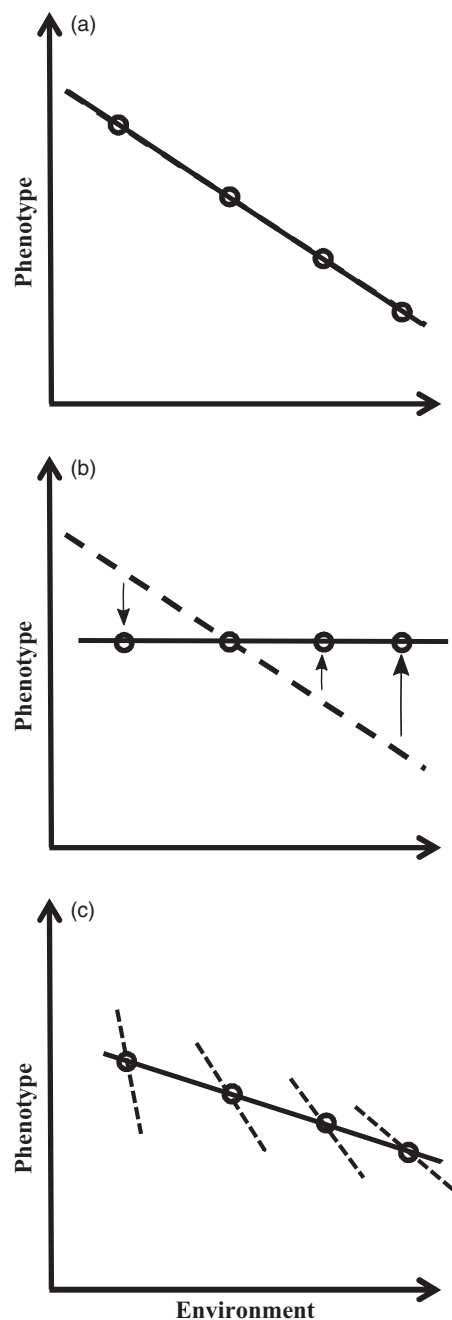


Fig. 1. Evolutionary scenarios to explain potential differences among populations in their plastic response. Each point represents the observed response of a population. Solid lines represent among population reaction norms; dashed lines represent population reaction norm. (a) Populations share a common reaction norm but exhibit local differences in their phenotypes as they are spread at different position on the environmental gradient. Among populations and populations reaction norms are confounded. (b) Populations share a common reaction norm but do not present any differences as selection promotes physiological or behavioural adaptations such that individuals breed on average at the same dates. (c) Each population evolves a locally adapted reaction norm influenced by local conditions.

When breeding phenology shows a linear relationship with environmental conditions, differences in phenotypic plasticity across populations may be assessed by analysing

the intercept and slope of the reaction norm (Baumann & Conover 2010; Baythavong & Stanton 2010; Husby *et al.*, 2010). The extent and the causes of variation in the slope of reaction norms for breeding date remain uncertain, but recent theory predicts that phenotypic plasticity should be greater, and therefore, the reaction norm should be steeper, in populations occupying sites characterized by large, but predictable, temporal environmental variation (Ghalambor *et al.* 2007; Chevin, Lande & Mace 2010). A comparative analysis of multiple populations inhabiting heterogeneous environments provides a unique opportunity to identify putative causal factors of selection on phenology plasticity (Husby *et al.*, 2010; Porlier *et al.* 2012; Klepsatel *et al.* 2013).

Squamate reptiles are ideal models to characterize reaction norms of breeding phenology because of their dependence of key physiological processes to body temperature (Huey & Stevenson 1979; Clusella-Trullas, Blackburn & Chown 2011; Le Galliard *et al.* 2012). Although the effect of climatic conditions on body temperatures may be buffered by compensatory responses of basking behaviour, most physiological performance traits of ectotherms are sensitive to climatic conditions, which in turn affects fitness (Huey, Hertz & Sinervo 2003; Huey *et al.* 2009; Kearney, Shine & Porter 2009; Sinervo *et al.* 2010; Clusella-Trullas, Blackburn & Chown 2011). Here, we analysed temporal and spatial variation in parturition date within and among populations of the common lizard (*Zootoca vivipara*). This lizard is an obligate hibernating species and all reproductive stages (vitellogenesis, ovulation/fertilization, gestation and energetic recovery) occur between early May and late September. Previous studies have demonstrated earlier parturition dates in warmer years (Chamaille-Jammes *et al.* 2006; Lepetz *et al.*, 2009; Le Galliard, Marquis & Massot 2010), but the interpopulation variation in reaction norms with respect to habitat variation has not yet been characterized.

In this study, we determined how environmental conditions affect parturition date using a sample of 11 populations from the Massif Central (France) that occupy an elevation gradient ranging from 1200 to 1450 m a.s.l. This set of populations includes variation in both climatic and anthropogenic conditions encountered by common lizards in the area. The region represents the southern margin of the distribution of the common lizard and local climatic conditions are heterogeneous depending on elevation and prevailing winds. Moreover, the region has a long history of pastoralism, and different levels of human-mediated alteration characterize our study sites. To estimate the impact of such heterogeneity on the reaction norm of parturition phenology, we addressed the following questions: (i) Is local variation in parturition date affected only by climatic factors? (ii) What is the thermal sensitivity of local reaction norms? (iii) How is variation in the reaction norms for parturition date structured according to prevailing weather and habitat conditions?

Materials and methods

NATURAL HISTORY OF *zootoca vivipara*

The common lizard *Z. vivipara* is a widely distributed lizard species in the family Lacertidae from Eurasia. Most of the European and Asian populations are ovoviparous except for egg-laying populations inhabiting the very southern and western margin of the distribution (Surget-Groba *et al.* 2001). In our study area, adult males emerge from hibernation in April, before yearlings and adult females. Females emerge in late April to early May. Mating happens immediately after females' emergence. Egg fertilization is initiated shortly after copulation and gestation occurs between the beginning of May and the middle of July. The onset of parturition may be as early as in mid-late July and last through early August. After parturition, females accumulate energy reserves until hibernation which starts on average in October (detailed life cycle and phenology in Bleu *et al.* (2013)).

POPULATION MONITORING AND BREEDING CONDITIONS

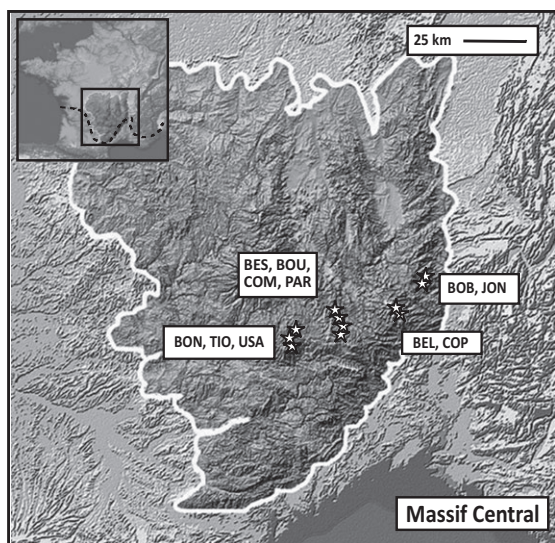
The focal populations are located in four mountain chains from the Massif Central, France (exact locations in Table 1 and map in Fig. 2). We sampled 11 populations during mid to late June every two years (i.e. an average of 5 capture episodes per population) from 2003 to 2014. However, some populations were sampled as few as 3 different years and others up to six different years (Table 1). The average date of capture was the 27th of June (SD = 3.5 days). The variation in date of capture was mainly due to poor weather conditions that decreased the efficiency of captures and delayed capture dates. At each sample period, we captured 15 to 20 pregnant females from each population and measured their snout to vent length (SVL, mean = 60.1 ± 4.9 mm) and body mass (mean = 4.76 ± 1.08 g). Pregnant females were brought to the laboratory and individually housed in a separate terrarium ($11 \times 18 \times 11$ cm) under standardized conditions (average temperature = 25.19°C ; SD = 2.31), until parturition (Massot & Clobert 2000). The average ambient temperature at each field site during the period of captivity was 22.2°C (SD = 0.83) but varied between 16.56°C (SD = 1.23) in the colder population and 24.81°C (SD = 3.21) in the warmer one. Females were exposed to the local prevailing photoperiod, but provided with access to heat for 6 h per day (two times for 3 h, once in the morning and one in the afternoon). Female mortality during captivity varied between 1 and 3% among years. Parturition date (PD) was calculated from the first of January (e.g. 1 = January 1st; mean PD = 199; SD = 7 days; or 18 July $\pm$ 1 week). Three days after parturition, each female was released with her offspring at the exact capture location. Over the 11 years of study, the females spent on average 22.4 days (SD = 6.9 days) in captivity. This period of captivity represents between 25 and -30% of the total gestation period that lasts about two and a half months.

DESCRIPTION OF LOCAL WEATHER DATA AND HABITAT QUALITY

Air temperature data for each population were obtained from the national French meteorological agency (MeteoFrance, MF, <http://publitheque.meteo.fr>). To explore the impact of temperature on parturition date, we used the mean maximal temperature

Table 1. Position and description of each population with associated mountain chain, sampling opportunities, elevation, forest cover index (FCI), meteorological station and estimated mean daily maximal temperature in June (T_{max6})

Name	Longitude	Latitude	Mountain Range	Number of samples	Meteorological station	Elevation, m	FCI	T_{max6}
BEL	44°40'3.32N	4°01'5.50E	Mont du Velay	7	Loubaresse	1385	0.30	18.82
COP	44°39'3.02N	4°01'7.82E	Mont du Velay	6	Loubaresse	1392	0.07	21.95
BES	44°35'2.81N	3°30'4.81E	Margeride	7	Mende	1200	0.1	21.63
COM	44°40'0.14N	3°31'9.14E	Margeride	6	Mende	1401	0.19	20.04
PAR	44°36'3.06N	2°29'4.79E	Margeride	6	Mende	1454	0.32	20.57
BOU	44°45'4.28N	2°28'9.05E	Margeride	5	Mende	1250	0.12	19.60
TIO	44°35'2.43N	3°06'3.37E	Mont d'Aubrac	6	Nasbinals	1272	0	17.96
USA	44°38'5.45N	3°07'2.33E	Mont d'Aubrac	4	Nasbinals	1219	0.05	15.85
BON	44°33'5.63N	3°07'6.47E	Mont d'Aubrac	6	Nasbinals	1344	0	17.18
JON	44°50'0.98N	4°12'6.49E	Mont du Vivarais	5	Mazzan Abbaye	1381	0.19	22.86
BOB	44°49'4.50N	4°13'6.96E	Mont du Vivarais	3	Mazzan Abbaye	1430	0.04	21.02

**Fig. 2.** Location of the common lizard populations in the Massif Central, France. The white line circumscribes the Massif Central. The dashed lines represent the southern margin of viviparous common lizard distribution. Localities of the study populations: BES = Lou Bes, BEL = Bel Air, BOB = Bout de la Barre, BON = Col de Bonnetcombe, BOU = Baraque du Bouvier, COM = Col du Cheval Mort, COP = Col du Pendu, JON = Gerbier des Joncs, PAR = Lou Paradis, TIO = Tioule and USA = Usanges.

for June (T_{max6}). First, June temperature has been shown as the best proxy of thermal conditions during gestation and is known to influence significantly the length of gestation in the common lizard (Chamaillé-Jammes *et al.* 2006; Lepetz *et al.* 2009; Le Galliard, Marquis & Massot 2010). Secondly, Lourdais *et al.* (2004) showed that the period of development the most sensitive to temperature is the middle part of gestation in the viviparous snake *Vipera aspis*. In the common lizard, this middle stage of gestation corresponds to June (Bleu *et al.* 2013). We estimated the value of T_{max6} that matched the period of time lizards were in the field at each study site and prior to capture. That is, we did not include data from days when females were in captivity.

We calculated local temperatures for each population from the closest MF meteorological station. However, we were unable to find a unique MF station for all populations (Table 1). As a consequence, we used temperature data from local conditions to

create T_{max6} data that corresponded with MF data. Our approach involved generating a calibration curve between MF values and local conditions. We deployed temperature data loggers (Thermochron iButtons[®], Waranet Solution, Auch, France) on the ground at each capture localities from mid June 2014 to the end of July 2014. We used linear regression to relate the temperatures recorded by the dataloggers for each population to those recorded by the nearest meteorological stations (see Fig. S1, Supporting information). We used the regression coefficients from the linear regression analysis to predict climatic conditions for each population from MF data (see details in Table S1). Despite the substantial altitudinal gradient present in our study, we did not use elevation in our statistical models because T_{max6} was a more relevant descriptor of breeding phenology variation than altitude (results not shown). We are not able to exclude the possibility that proxies other than T_{max6} are better in some populations but the fit of our reaction norms was satisfactory in most cases and this approach allows us to use a common model to analyse variation among the eleven populations. Finally, we also calculated an index of Forest Cover (FCI) to assess the effects of anthropogenic activities and thus local habitat quality. In the study populations, the primary source of disturbance is grazing by livestock. Using aerial photographs (scaled Google Earth[®] views, Mountain View, CA, USA), we measured FCI as the proportion of pixels representing trees or bushes within the total capture area.

AMONG-POPULATION VARIATION IN REACTION NORMS

To study reaction norms of parturition dates (PD) across populations, we performed a three-step analysis. First, we tested whether geographical proximity between populations was important by nesting each population within their individual mountain chain in a mixed regression model with population as a random factor. The goodness-of-fit of this model was then compared using the corrected Akaike Information Criteria (AICc) to a model excluding the effect of local mountain chain and the null model. Secondly, using a linear mixed regression models, we tested patterns of variation in the reaction norms among populations. The full model included fixed effects of T_{max6} , T_{max6}^2 (to account for a potential nonlinearity), FCI, female SVL (as in previous studies) and first-order interactions between temperature variables and FCI. All variables were centred and scaled by their standard deviation to facilitate the interpretation of model estimates. We

searched for both interpopulational differences in average value of parturition date (intercept of the reaction norms) and in the response to variation in T_{max6} (steepness of the slope) by including random effects of population on the intercept only or on both slope and intercept of the model:

Minimal model:	$PD \sim \text{fixed effects}$
Random intercept:	$PD \sim \text{fixed effects, random} = \text{Population}$
Random intercept and slope:	$PD \sim \text{fixed effects, random} = \text{Population} + T_{max6} * \text{Population}$

We first evaluated the random part of the model using AICc selection and a likelihood ratio test (LRT). Then, we assessed the significance of fixed effects using backward selection of non-significant terms (threshold of significance <0.05 , Zuur *et al.* 2009).

Last, given significant variation in the thermal reaction norms (see below), we assessed whether the position of each population along the thermal and habitat gradient influenced the intercept and slope coefficients. We retained the regression coefficients of a linear model linking parturition date to T_{max6} for each population after temperatures were centred on the mean temperature of the eleven populations. Coefficients of the reaction norms were then regressed against the local mean T_{max6} (mean value from each population), FCI and variance in T_{max6} (VT_{max6}) using multiple linear regression models. We used two variables to represent two different aspects of climatic conditions: T_{max6} represents mean ambient temperature conditions during mid-gestation for each population, whereas VT_{max6} captures the temporal heterogeneity of weather conditions during the 11 years of sampling. However, their values were positively correlated (Spearman correlation coefficient, $r = 0.574$). To check for some collinearity issues, we calculated both Pearson's bivariate correlations predictor ($|r| = 0.67$) and the variance inflation factor ($VIF = 2.00$). These values are below the traditional thresholds ($|r| < 0.7$ and $VIF < 5$, respectively) suggesting no significant collinearity between the two variables. Hence, we retained both variables to determine the impact of climatic conditions on the shape of the reaction norms and employed backward elimination of the non-significant variables to select for the best model.

Results

Average T_{max6} (the mean maximal temperature of June) during the sample period 2003–2014 varied from 15.85 °C to 22.86 °C. Our index of disturbance, FCI, varied from 0% forest cover (fully grazed habitat) to 32% (mesic, mixed open habitat, see Table 1). Average parturition date (PD) varied between day 195 and 203 across populations and years. We found no evidence of a trend towards warmer conditions during the time period of this study (10 years); instead, all populations showed a weak decrease of temperature between 2003 and 2014 (Fig. S1). Females captured from populations located in the 'Mont d'Aubrac' mountain chain had earlier parturition dates despite experiencing cooler ambient temperatures (Fig. 3). However, according to ΔAIC differences, the model with a random population effect ($AICc = 6638.8$) outperformed both the nested model with a mountain chain effect ($AICc = 6782.3$) and the null model ($AICc = 6713.9$). Thus, the population differences over-

ride the regional influence among mountain chains. Based on this result, we used the model with a random population as a starting point in our analysis of the reaction norms.

The linear mixed models showed significant interpopulation differences in both the mean and slope of the reaction norm ($AICc$ minimal model = 6472.5, $AICc$ random intercept model = 6134.1, $AICc$ random intercept and random slope model = 5986.1). Selection of fixed effects led to a final model summarized in Table 2. First, females with a larger SVL had earlier parturition dates (slope $b = -0.91 \pm 0.15$; $t_{1032} = -5.98$; $P < 0.0001$). In addition, PD was significantly advanced by T_{max6} ($b = -5.88 \pm 2.54$; $t_{1032} = -2.31$, $P = 0.021$) and was influenced by a two-way interaction between T_{max6}^2 and FCI (Table 2). The exact pattern was that T_{max6}^2 decreased PD when FCI equals zero ($b = -2.92 \pm 0.41$; $t_{1032} = -7.12$; $P < 0.0001$), but PD is delayed by FCI when T_{max6}^2 equals zero, that is climate conditions are average ($b = 4.94 \pm 2.19$; $t_9 = 2.25$; $P = 0.050$). In addition, there is a reduced effect of FCI on the delay of PD at higher values of air temperatures in June ($b = -1.41 \pm 0.62$; $t_{1032} = -2.19$; $P < 0.023$).

We estimated local reaction norms by performing a linear regression between PD and local temperature for each population (see Table S1, for details of regression estimates). All reaction norms exhibited a negative relationship between mid-gestation temperatures and PD (Fig. 3). The one exception was BOB, which had a positive association. We found a significant and positive effect of VT_{max6} on the slope of the reaction norms ($b = 2.281 \pm 0.83$; $t_8 = 2.74$, $P = 0.0254$; Fig. 4e). We also found a significant and positive effect of T_{max6} on the elevation of the reaction norm ($b = 5.89 \pm 0.95$; $t_8 = 6.190$; $P = 0.0002$; Fig. 4b). One notable pattern is the reaction norm for USA. This population is characterized by particularly cold climate conditions and its reaction norm was a significant outlier. We tested whether USA influenced the relationship between coefficients of the reaction norm and environmental conditions by reanalysing the data without USA. However, the qualitative and quantitative pattern remained unchanged with the exclusion of USA (see details Fig. 4).

Discussion

In the present study, we confirmed, at a broad geographic scale, that populations of the common lizard advanced their parturition date (PD) in years with warmer temperatures during the mid-gestation period (June). We also found that PD is influenced by the interaction between variability in climatic conditions and anthropogenic disturbance as assessed by the extent of grazing. Finally, we demonstrated that PD reaction norms vary among several common lizard populations and that differences in both the mean and sensitivity of PD to thermal conditions are correlated with local climatic conditions.

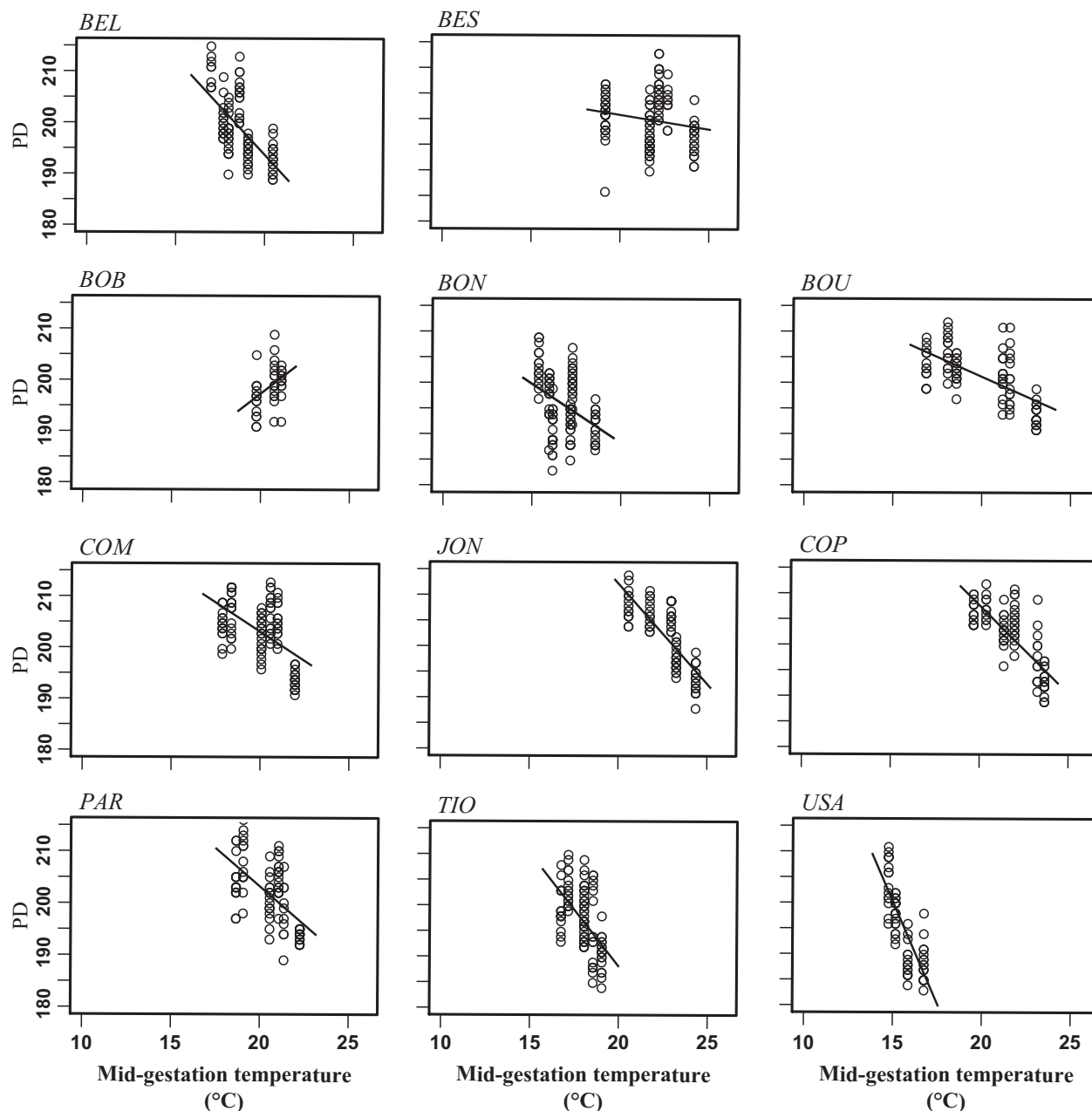


Fig. 3. Population-level reaction norms in each study site of global survey, described as the relation between parturition date and average temperature in mid-gestation (T_{max6}). All relationships were significant ($P < 0.05$; with the exception of BES $P = 0.06$). We excluded one population from the analysis (BOB) as the relation was positive, which may reflect insufficient sampling opportunities.

IMPACT OF LOCAL CONDITIONS ON PHENOLOGY

Parturition dates of *Z. vivipara* in the Massif Central were generally earlier when females experienced warmer temperatures during mid-gestation. This pattern was evident across all populations in Massif Central and is consistent with previous studies based on a single population on Mont Lozère, which showed that females have specific thermal requirements for gestation during June that are more difficult to attain in cooler years (Chamaille-Jammes *et al.* 2006 and Le Galliard, Marquis & Massot 2010). In our study, we extended this result by showing that the

local climate supersedes regional climate conditions when modelling phenological reaction norms across populations at a broad geographic scale. Indeed, some populations included in our sample were spatially clustered in mountain regions and sometimes only few kilometres apart (Fig. 2). However, local conditions, and in particular thermal conditions, were stronger determinants of PD than spatial proximity. Furthermore, we detected the effects of climate and anthropogenic disturbance on PD even though females were maintained in captivity during the last third of their gestational period. The warm and homogeneous laboratory conditions may have introduced

Table 2. Best linear mixed effects model summary table for parturition date vs. snout to vent length (SVL), forest cover index, mid- and squared mid-gestation conditions (T_{max6} , T_{max6}^2) and first interaction terms. Temperature was nested within populations as a random effect

Predictor Variables	Estimate	SE	d.f.	t-value	P-value
Intercept	196.96	3.88	1032	50.74	<0.0001
SVL	-0.91	0.15	1032	-5.99	<0.0001
FCI	4.94	2.19	9	2.26	0.050
T_{max6}	-5.89	2.55	1032	-2.31	0.006
T_{max6}^2	-2.93	0.41	1032	-7.13	0
CFI * T_{max6}^2	-1.41	0.62	1032	-2.27	0.042
Random effects	SD	Corr			
Population (intercept)	12.79				
Population (slope)	4.52	0.84			

a bias in our estimation of PD reaction norms through buffering differences among individuals and populations. For example, such conditions would be expected to result in more homogeneous patterns of the timing of parturition. Yet, we recovered significant variation in PD among individuals and populations that is consistent with local climate conditions. Thus, the sampling of females during the latter period of gestation (when thermal effects are less critical, Lourdaix *et al.* 2004) may have resulted in

our underestimating the magnitude of plasticity in breeding phenology.

Our results also show that anthropogenic activities have a direct effect on the breeding phenology but also an effect in relation with the effect of ambient temperature. This is suggested by the significant interaction between local temperature and the forest cover index. The interaction between these factors exacerbates the effects of ambient temperatures during mid-gestation and may be a consequence of differences in the ability of common lizards to cope with temperature in fully open vs. partially covered habitats. Our analyses suggest stronger effects of temperatures in open, that is, heavily grazed areas. Economic activity in the Massif Central is, in large part, dependent on meat and milk production, pastoralism and agriculture. In the next few decades, the increase in temperatures and the new economic dynamic in the region by promoting livestock production hence grazing activities should lead to an increasingly disturbed and open landscape, enhancing the effects revealed in our analyses. Moreover, different species of livestock (e.g. cows, sheep, horses) do not pose a direct threat to lizards, but may have severe indirect effects by disturbing basking behaviour, reducing the availability of thermal refuges or by carrying parasites that may affect lizard performance.

It is difficult to assess the fitness consequences of accelerating embryogenesis because shifts in parturition date

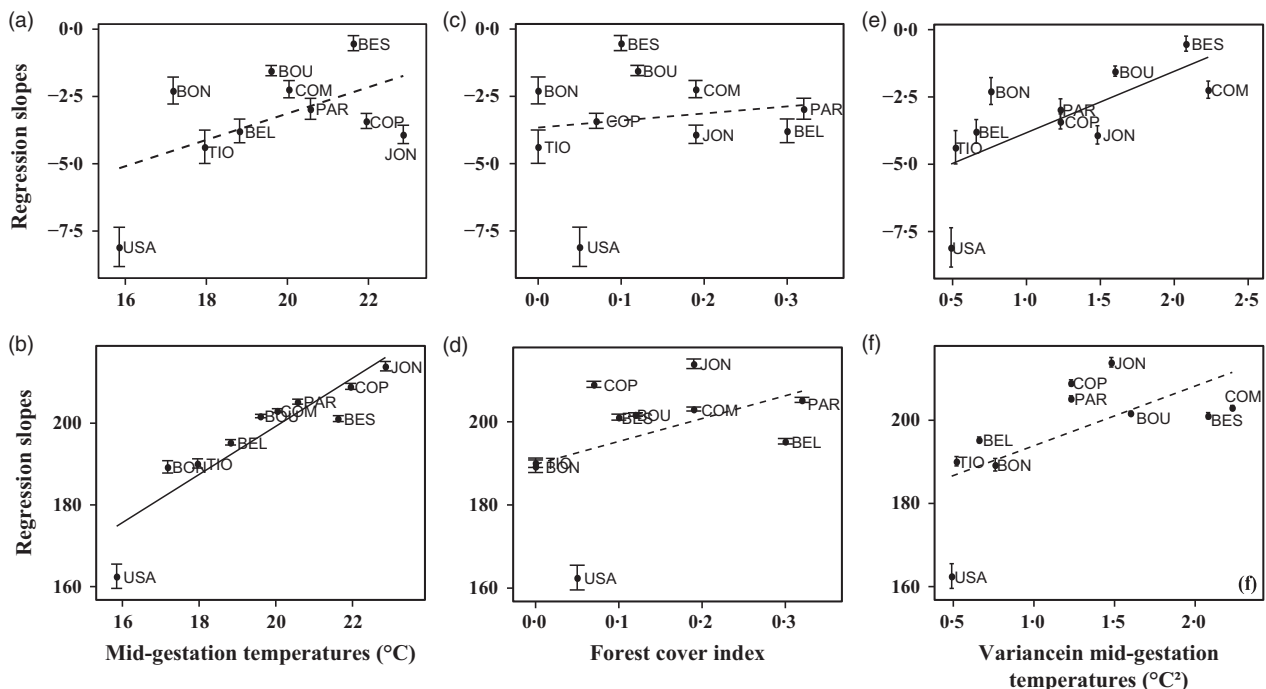


Fig. 4. Slope of the population reaction norms ('Regression slope'; a., c., e.) and intercept ('Regression intercept'; b., d., f.) against temperature during mid-gestation (a. and b.), Forest Cover Index (c. and d.) and Variance in Mid-gestation temperatures (e. and f.). Solid lines represent significant relationships ($P < 0.05$); dashed lines represent non-significant relationships. We note that the USA population may drive observed relationships. However, the population experiences cooler environmental conditions than other population, because of the constant windy. We hypothesize that this extreme environment is relevant to consider when comparing phenotypes among different. However, we repeated our analyses with USA removed from the data set and the results remained unchanged (The slope of the reaction norms remain correlated with VT_{max6} , $P = 0.034$; and the elevation is still influenced by T_{max6}^2 , $P = 0.004$).

can have either positive or negative consequences on fitness components in common lizards. On the one hand, earlier PD should enhance the growth rate of neonates Bestion *et al.* 2015 and increase offspring survival (Le Galliard, Marquis & Massot 2010), as well as maternal survival (Chamaille-Jammes *et al.* 2006). Yet, a shorter gestation period is also linked with warmer temperatures that, if associated with drought, could be lethal for neonates, as experimentally demonstrated by E. Bestion, A. Teyssier, M. Richard, J. Clobert, J. Cote (in preparation). All things considered, we can expect a mixture of habitat and climatic factors to not only impact phenology as shown in our analysis but to affect population dynamics and age structure (Townroe & Callaghan 2014), individual parasite loads (Pounds *et al.* 2006) and species distribution (Krockenberger, Edwards & Kanowski 2012).

The negative quadratic effect of temperature during gestation on mean parturition date across populations suggests an accelerating effect of thermal conditions on the speed of gestation. High body temperature is known to enhance physiological performance in ectotherms up to a given temperature threshold, called optimal temperature (T_o) for performance (Huey & Stevenson 1979). This non-linear sensitivity to temperature could be one hypothesis to explain the pattern of accelerating progressively gestation timing. Because sampling opportunities in our study system was limited to a maximum of six parturition years, it is challenging to disentangle whether the observed non-linearity was due to nonlinear intrapopulation plasticity rather than differences in reaction norm slopes among populations (see Fig. 3). Detailed data from a long-term study of one population of *Z. vivipara* on Mont Lozère monitored by capture–recapture protocols continuously from 1986 to 2012 (see Massot, Clobert & Ferrière 2008 for a complete description) offered a unique opportunity to test for the existence of a quadratic, decelerating reaction norm in the same population. Supplementary analyses based on this population showed that the temperature during gestation ranged from 18.4 to 25.1 °C, but that parturition date was linearly, and not quadratically, related to mid-gestation temperature in this population (see Fig. S3 and Table S4). This result suggests that the nonlinearity observed in our large sample of populations may be a consequence of differences among populations rather than a truly, nonlinear plasticity (see next section).

AMONG-POPULATION VARIATION IN REACTION NORMS

One goal of our study was to explore the environmental characteristics that shape among-population variation in the reaction norm for parturition date including mean thermal conditions, variance in thermal conditions and forest cover. We found that consistent exposure to warm temperatures a heterogeneous pattern of advanced parturition dates depending on local weather conditions.

In contrast with theoretical predictions (Ghalambor *et al.* 2007; Chevin, Lande & Mace 2010), parturition dates decreased more quickly with local temperature in populations characterized by a lower variance in mid-gestation thermal conditions. These results are surprising as they show a significant negative relationship between the magnitude of the plasticity and environmental heterogeneity. In other words, sites with low interannual variation in mid-gestation conditions show the strongest response to climate (greatest negative slope of the reaction norm), whereas sites with higher variation display weaker responses (flatter reaction norms; see Figs 3 and 4). This result is of particular importance in the context of current global change, which may enhance variations in weather conditions and alter environmental stability (IPCC 2013). Indeed, an increased instability of climatic conditions may lead populations that are used to stable conditions (and that present the strongest reaction norms) to produce disproportionate or maladapted responses (Ghalambor *et al.* 2007). Given enough time, those populations may shift to flatter reaction norms through behavioural, physiological or genetic adaptation. Alternatively, failure to adjust to new environments may result in local extinctions (Sinervo *et al.* 2010).

The observed divergence in the magnitude of the plastic response may partially be explained by the predictability of ambient conditions during mid-gestation rather than the variance *per se*. The few studies that have compared PD reaction norms between different populations of a same species (Lind & Johansson 2007; Husby *et al.* 2010; Porlier *et al.* 2012) and some theoretical models (Ghalambor *et al.* 2007; Chevin, Lande & Mace 2010) suggest that unpredictable environments will impede the evolution of plasticity as the probability to produce a maladapted (or miss-matched) response is enhanced when heterogeneity is too great. In our case, environments with higher levels of variability in mid-gestation temperatures may be less predictable; however, it is difficult to statistically assess the predictability of the climate with available data and we cannot confirm that predictability is the factor that drives the relation. A second hypothesis might be that females are more responsive in colder populations. For example, populations with the least variable thermal conditions during gestation tend to be the coldest in our data set (Fig. 3e, marginal correlation: $P = 0.11$). The observed differences could thus reflect local adaptation that enhances the ability of females to benefit from advantageous thermal conditions when they occur. Indeed, a shortened gestation period may have been selected to release the pressure on the post-gestation period that drives preparation to hibernation of the adults and the juveniles (E. Bestion, A. Teyssier, M. Richard, J. Clobert, J. Cote, in preparation).

In conclusion, our results (i) provide evidence of interactive effects of anthropogenic disturbance and climatic conditions on parturition date, (ii) suggest that variation in PD reaction norms is correlated with local environmen-

tal conditions at a broad geographical scale and (iii) indicate that local climate variation is a major driver of spatial variation in breeding phenology plasticity. Altogether, these results emphasize the need of considering multiple factors that could drive local adaptation of reaction norms (i.e. temperature, human activities) when trying to assess how local adaptation may help populations to cope with climate change. Our study also underlines the need to take into account variation in reaction norms in the management strategy of populations threatened by climate warming and caution against the use of stable thermal reaction norms to predict the future ranges of species with climate niche models.

Acknowledgements

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Authors' contributions

All authors contributed to acquisition of field data; AR carried out the statistical analysis and drafted the manuscript; DBM, JFG and JC contributed to statistical analysis and interpretation of data; MR, DBM, JFG contributed to writing the manuscript. AR, JC and BS designed the study. All authors gave final approval for publication.

Data accessibility

Field data available from the Dryad Digital Repository: <http://doi.org/10.5061/dryad.qd5gj> (Rutschmann *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Air temperatures during mid-gestation conditions for each population in the study.

Figure S2. Regression between Meteo-France (MF) temperature and the temperatures obtained from iButton® data loggers deployed at each study site.

Figure S3. Relation between Parturition date (PD) and mid-gestation conditions on the long term capture mark recapture study site ROB. Despite an accelerating effect at the inter-population level, the relation does not significantly exhibit such an acceleration at the population level.

Table S1. Characteristics of each population.

Table S2. Predictor coefficients for a mixed effect model confronting parturition date (PD) to snout-vent length (SVL), mid-gestation temperature (T_{max6}), squared mid-gestation temperature (T_{max6}^2) on a 23 years old dataset.

Auteur

Alexis Rutschmann

Titre

Variations environnementales et plasticité phénotypique chez le lézard vivipare (*Zootoca vivipara*): une approche multi-échelle.

Directeurs de Thèse

Dr. Jean Clobert, Dr. Murielle Richard & Pr. Donald Miles

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Résumé

Les changements globaux récents ont des conséquences multiples et parfois dramatiques sur les communautés animales et végétales. La plasticité phénotypique permet aux individus de limiter ces effets par une transformation rapide de leur phénotype en fonction des conditions du milieu de vie. Cependant, l'intensité et l'efficacité de la réponse plastique sont largement influencées par plusieurs types de contraintes (environnementales, physiologiques et/ou génétiques) et on peut s'attendre à observer des variations de cette réponse entre populations et entre individus. Les espèces ectothermes, qui dépendent de la température de leur environnement pour tout activité physiologique, sont d'excellents modèles pour mener une approche intégrative du rôle de ces limites dans l'évolution de la plasticité phénotypique. Au cours de ce travail de thèse nous avons donc étudié les variations et le rôle adaptatif de plusieurs réponses plastiques du lézard vivipare (*Zootoca vivipara*) telles que la date de parturition ou l'investissement reproducteur. Par ailleurs, nous avons mené ce travail à plusieurs échelles spatiales et temporelles, à différents niveaux d'intégration (de la métapopulation au génome) et en utilisant une large gamme de méthodes (suivi à long terme, génétique quantitative et approches expérimentales).

Mots-clés : Plasticité phénotypique, normes de réaction, changement climatique, plasticité adaptative-non adaptative, *Zootoca vivipara*

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Laboratoire d'accueil

Station d'Ecologie Théorique et Expérimentale
2 route du CNRS
09200 Moulis