Local adaptation of a *Drosophila* parasitoid: habitat-specific differences in thermal reaction norms.

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Abstract

Local climate is an important source of selection on thermal reaction norms that has been well investigated in cline studies, where populations sampled along altitudinal or latitudinal gradients are compared. Several biotic factors vary with climate but are rarely integrated as alternative agents of selection to climatic factors. We tested the hypothesis that habitat may select for thermal reaction norms and magnitude of phenotypic plasticity in a drosophila parasitoid, independently of the climate of origin.

We sampled populations of *Leptopilina boulardi*, a *Drosophila* parasitoid in two different habitats, orchards and forests. Orchards offer laying opportunities over small distances for parasitoids, with a low variability in the number of hosts per patch, while forests offer more dispersed and more variable patches. The sampling was realised in a temperate and a Mediterranean climate. We measured egg load, volume of eggs, longevity and lipid content for parasitoids reared at two temperatures.

Reaction norms were opposite for populations from forests and orchards for investment in reproduction, independently of the climate of origin. The maximal investment of resources in reproduction occurred at the lower temperature in orchards and the higher temperature in forests. Host distribution differences between habitats may explain these opposite reaction norms. We also observed a flatter reaction norm for egg load in forests than in orchards. This relative canalization may have been selected in response to the higher variability in laying opportunities observed in forests. Our results demonstrate the potential role of resource distribution in evolution of thermal plasticity.

Keywords: climate, drosophila parasitoid, environmental canalization, genetic differentiation, *Leptopilina boulardi*, life history traits, phenotypic plasticity, temperature.

Introduction

According to the theory of evolution, natural selection should drive organisms to an optimal combination of life history traits in a given environment (Roff 1992). In ectotherms, the evolution of life history traits in response to temperature has been well investigated as this factor directly affects their fitness. To study the effect of temperature, authors have developed several approaches such as selection in the lab at different temperatures over several generations (e.g. Huey et al. 1991, Partridge et al. 1995), or clinal studies. By comparing populations or species originating from different climates and reared in a common garden (i.e. common temperatures), authors have observed clinal pattern for the great majority of traits in various taxa such as an increase in cold resistance in insects and gastropods (Addo-Bediako *et al.* 2000, Hilbish 1981) and growth rate in amphibians and fishes (Laugen *et al*. 2003, Yamahira & Conover 2002) in populations/species from cold climates, or an increase in heat resistance in insects from hot areas (Hoffmann *et al.* 2002, Sørensen *et al.* 2005). Schmidt *et al.* (2005), Schmidt & Paaby (2008) and Mitrovski & Hoffmann (2001) have observed that reproduction of *Drosophila melanogaster* females from colder environments was delayed compared to females from hotter areas while they lived longer. These studies have shown that in a common garden, organisms from different climates exhibit locally adapted life history traits and these differences are genetically determined.

Genetic variation in phenotypic plasticity of life history traits also exists along thermal clines. Climate may select for the thermal range and the slope of the reaction norm (i.e. a function with the value of the environmental variable as argument and the genotypic value of the trait as function value, de Jong 1990), which gives information on the level of phenotypic plasticity. There are two main assumptions. The first one is a shift of the

reaction norm toward higher temperatures in warm-adapted populations or toward lower temperatures in cold-adapted populations, such as found in *D. melanogaster* populations for wing and thorax length (Morin *et al.* 1999) or offspring production (Trotta *et al.* 2006). The second theoretical assumption, which has been rarely investigated, is a higher level of phenotypic plasticity in morphological and physiological traits (Bradshaw 1965, de Jong 1995) and canalization (i.e. an absence of plasticity or at least a lower level of plasticity) in fitness-related traits in variable climates, as these traits should be buffered against environmental variation (Stearns and Kawecki 1994, Wagner *et al.* 1997). Liefting *et al.* (2009) have observed that developmental rate, a fitness trait, of *Drosophila serrata* was more canalized in populations from temperate climate (i.e. a variable climate) than tropical climate (i.e. a stable climate) in response to different temperatures, whereas morphological traits were more plastic in temperate climate.

Liefting & Ellers (2008) also observed habitat-specific differences in growth rate thermal plasticity by comparing springtail populations inhabiting close forests and heathlands. The authors found flatter thermal reaction norms in heath populations compared to forests populations. It is likely that microclimate explained these differences, as temperature and other climatic parameters are more variable in heathland. Most studies only consider climate as a selective force on thermal reaction norms in cline studies, but the environment experienced by an organism is the combination of many interacting biotic and abiotic factors. Other parameters than temperature vary with climate, such as interacting species (Kraaijeveld & Godfray 1999, Fleury *et al.*, 2004) or nature and distribution of available resources (Ellers & van Alphen 1997), and may potentially affect evolution of thermal plasticity. In parasitoids, fitness is directly dependent on the number of hosts parasitized.

Host distribution, varying with climate, may thus be a major force in the evolution of life histories of parasitic wasps, even stronger than climate, as suggested by Moiroux *et al.* (2010). They found strong differences in life history traits between populations of parasitoids originating from contrasting climates, but temperature of origin did not explain these differences. They suggested that life histories have been selected mainly in response to the spatial and temporal distribution of hosts and not climatic factors, but the absence of data on host distribution did not allow the authors to provide evidence for this hypothesis.

In this study, we tested the hypothesis that temperature-induced plasticity in fitness traits may have differently evolved between habitats mainly characterized by different host distribution. We compared life histories of females of a *Drosophila* parasitoid, *Leptopilina boulardi*, from two different habitats with particular host distribution: orchards and forests. During the fruiting season, orchards offer nearby laying opportunities with low variability in the number of hosts per patch while the number of hosts per patch and the distance between them are more variable in forests, where *Drosophila* lay their eggs on scarce fruits or sapfluxes on wounded trees. These habitat differences were investigated in a temperate and a Mediterranean area with two contrasting climates. We measured fecundity and longevity thermal reaction norms as they are major components of fitness and a trade-off generally occurs between these two traits in parasitoids (e.g. de Jong & van Noordwijk 1992, Ellers 1996). Lipid content thermal reaction norm was also studied as these nutrients represent the main and limited energetic resources allocated to survival and/or reproduction in parasitoids (Rivero & Casas 1999, Ellers & van Alphen 1997). Moreover we tested for genetic differentiation between orchards and forests using AFLP to ensure that populations evolved separately in different habitats and may thus be locally adapted. If populations

were not genetically distinct, two genotypes with different habitat preferences may occur in a same population, as found in Drosophila (Taylor & Powell 1976). We expected to find similar reaction norms between similar habitats sampled in both climate and different reaction norms between habitats from a similar climate.

Material and Methods

Leptopilina boulardi (Hymenoptera: Figitidae) is a solitary endoparasitoid that mainly attacks *Drosophila melanogaster* and *D. simulans* larvae (Diptera: Drosophilidae). This species occurs in the Mediterranean Basin, in tropical Africa, in North and South America and in the West Indies (Fleury *et al.* 2009).

Sampling and Field data

We sampled four populations of *Leptopilina boulardi* near the France-Spain border in orchards and forests in two contrasting climates. Populations were sampled in 2007 in Casteil (Pyrénées-Orientales, South of France; 42°31’55’’N, 2°23’39’’E) and Calonge (Cataluña, North of Spain; 41°51’45’’N, 3°04’35”E), which have different climates (table 1). Casteil is located in Pyrenean Mountains where winters are cold and snowy and summers are sunny. There, we installed traps in apple trees in four close orchards in the village and in a forest of broadleaf trees, at four to five kilometres of the village.

Calonge is a coastal city located 100 km north-east of Barcelona, with a Mediterranean climate (mild winters, very hot summers, little precipitation). Traps were installed in four

orchards (apple and peach trees) in the city, and in a Mediterranean forest (mainly cork oaks) at eight to ten kilometres from the orchards, in direction of the land.

In both climatic regions, the sampling was realised in September, respectively a few days before and after the harvest in France and Spain, when many apples are decaying on the ground and drosophila are thus very abundant in orchards. Twenty banana bait traps (i. e. a plastic container with a 3 cm diameter hole covered with a mesh with 3 mm openings) were used per site. In the field, we counted the number of fruit flies captured per trap – used as a proxy of hosts available for parasitoids- and we measured the host inter-patch distance (i.e. the distance from the trap to the closest patch with active drosophila) to estimate host availability. In the lab, fruit flies and parasitic wasps emerging from traps were also counted. Thirty females per population were randomly taken from the offspring to set up lab cultures. *Leptopilina boulardi* was the only parasitoid found at this time in our traps in all locations.

Preliminay tests

L. boulardi was considered as a proovigenic parasitoid (i.e. females mature all their eggs before the start of their adult life, Kopelman & Chabora 1986) unable to synthesize lipids during adult life, but Moiroux *et al.* (2010) found synovigeny (i.e. additional eggs are matured during adult life) and lipogenesis ability in some Iranian populations. We checked for these characters in our four populations but there was no evidence that synovigeny or lipogenesis ability occur in Pyrenean populations (appendix 1). Thus, egg load at emergence corresponds to the total fecundity.

Moreover, we checked for the presence of Lbfv viral particles described by Varaldi *et al.* (2003) in some *L. boulardi* populations and responsible for an increased tendency to superparasitize and changes in several life history traits in infected females, such as fecundity or locomotor activity (Varaldi *et al.*, 2005). We did not detect any evidence for viral infection in any population. It is thus unlikely that this virus explains differences in life histories (appendix 2).

AFLP analysis

As populations from orchards and forests were collected only a few kilometres apart, we studied if orchard and forest populations did belong to genetically distinct populations, using amplified fragment length polymorphism technique (AFLP) as neutral characters.

From the wasps emerging in the lab from banana bait traps, thirty females and males per population were isolated in Eppendorf tubes, frozen in liquid nitrogen and conserved at -80°C for genetic analyses. The AFLP procedure was performed according to the standard protocol described by Vos *et al.* (1995) with slight modifications (Appendix 3). We used eight combinations of primers for the selective PCR.

Life history traits

Rearing. *Drosophila melanogaster* used as hosts in the cultures originated from strains collected in the Netherlands in 1960. *Drosophila* larvae used for parasitoid cultures were laid at 25°C during a two-hour period in a baker's yeast suspension. That short time allowed us to limit variation in host size that would be induced by a too long period between first and last oviposition. For our experiment, ten females *L. boulardi* oviposited in separate

jars at 25°C for 24h in 100 second instar *D. melanogaster* larvae in a baker's yeast suspension. The parasitized larvae were then separated in two groups and placed at 20 °C or 25°C. At emergence, three females from each jar were placed in Eppendorf tubes, frozen in liquid nitrogen to stop instantaneously any metabolic activity and conserved at –80°C. These females (i.e. 3 females per mother for each temperature) were then used for measurements of egg load, volume of eggs, lipid content and dry mass (i.e. 30 females tested per population per temperature). The remaining females emerging from the jars were equally separated at 20 or 25°C and used for measurement of longevity (per temperature: France forest, n=37, France orchards, n=51, Spain forest, n = 43, Spain orchards, n=25).

Egg load and volume of eggs. Females were placed in a drop of Ringer's solution on a microscope slide and dissected under a binocular microscope (×40, Olympus SZX9). We removed both ovaries and counted eggs while the rest of the female's body was conserved for later lipid extraction. After counting, eggs were photographed (Olympus Camedia C3040) under a microscope (×4, Olympus BH2). Length (L) and width (w) of 30 eggs per female were measured with the numeric image analysis software AnalySIS to calculate egg volume (taken as a prolate spheroid volume: $V=4/3\pi Lw^2$).

Lipid content. Lipid quantity of the females was measured after removing eggs, using the protocol proposed and successfully used by Vernon and Vannier (1996) and Terblanche *et al.* (2004). Wasps were dried at 40 °C during four days in an air oven and weighed to measure dry mass (Sartorius M4 ±0.001 mg). They were then left during two weeks in an Eppendorf tube containing 1 ml of chloroform/methanol solution (2:1) to extract lipids.

Females were then placed at 40°C during 24 hours after extraction and weighed again to deduce lipid quantity and lipid content (=lipid quantity/lean dry mass).

Longevity. Adult parasitoids were reared at the developmental temperature (20°C or 25°C), 50% RH, 12L: 12D in glass jars on an Agar-Nipagine substrate and were fed *ad libitum* with diluted acacia honey. Substrate was renewed every week. Dead individuals were counted and removed twice a day, each morning and evening.

Statistical analysis

AFLP analysis. The general matrix of presence/absence of DNA fragments was analyzed with the free software AFLP-Surv 1.0. That software estimates genetic diversity and population genetic structure using the Lynch & Milligan (1994) approach for diploid organisms, assuming an Hardy-Weinberg equilibrium. 1000 permutations were performed to calculate Hj (Nei's gene diversity), Fst and test for genetic differentiation between populations.

Life history traits. We performed generalized linear models with habitat as a paired fixed factor, temperature and climate region as fixed factors, and dry mass as a covariate to test for differences in egg load, volume of eggs and lipid content reaction norms. We considered a Poisson distribution for egg load while egg volume and lipid quantity were normally distributed. Dry mass, measured before lipid extraction, was integrated as a covariate to correct life history traits for the body size.

We used a Cox model with habitat as a paired fixed factor, temperature and climate region as fixed factors, and dry mass as a covariate to test for differences test for differences in longevity between populations.

All the statistical analyses were carried out using R software version 2.14.1 (R Development Core Team, 2011).

Results

Field data:

In the field, more *Drosophila* of the *melanogaster*-group (*D. melanogaster* and *D. simulans*) were captured in orchards than forests ($\chi^2 = 47.59$, $p < 0.001$), and the inter-patch distance was smaller in the first habitat ($\chi^2 = 13.31$, $p < 0.001$). Fruit trees and *Drosophila* distribution were more homogeneous in orchards (i.e. a small variance in distance between trees and in number of *Drosophila* captured per trap) with rich patches (180 ± 30 apples per tree in France, 165 ± 35 in Spain), whereas host distribution was more stochastic (i.e. a high variance in number of *Drosophila* captured per trap and in distance between laying opportunities) in forests and patches were on average poorer (table 1). However we found in both French and Spanish forests some wild apple trees that represent high-quality patches (40 ± 15 apples per tree in France and Spain) for this habitat. Moreover, the ratio of Drosophila found per trap relative to the number of wasps, and hence interspecific competition, was similar in forests and orchards (Table 1).

AFLP analysis

770 loci were scored in this study and 641 of them were polymorphic. Our results indicate that we had similar genetic diversity in the four populations and that genetic differentiation occurs with the global data set (Fst = 0.08, p = 0). When testing separately the two regions, we observed genetic differentiation between orchards and forests in France (Fst = 0.062, p = 0.034) and Spain (Fst = 0.077, p = 0.027) despite distances less than 10 km.

Life history traits

We found significant main effects of habitat and rearing temperature on egg load, lipid content and longevity, with dry mass as a covariate (Table 2). The climatic area of origin (temperate vs Mediterranean) generally did not explain differences between populations. Females from forests invested more in reproduction, had a lower lipid content and lived shorter than females from orchards at 25˚C, while no difference occurred between populations at 20˚C except for longevity, forests females living slightly longer than orchards females.

The reaction norm slopes were opposite for investment in reproduction (i.e. egg load corrected for body size) between females from different habitats, independently of the climate of origin (significant temperature x habitat interaction in Table 2): females from orchards had significantly more eggs at 20°C than 25°C for a given size, whereas females from forests had more eggs at 25°C than 20°C (figure 1). The reaction norm of egg load was flatter in forests than in orchards (figure 3a).

The slopes were also different for lipid content as it decreased between 20 and 25°C in forests, whereas no difference was detected between temperatures in orchards (figure 3b).

Longevity decreased as temperature increased in all populations but reaction norms were flatter in females from orchards.

We did no detect any effect of habitat, temperature or dry mass on the volume of eggs.

Discussion

In this study, we tested the hypothesis that temperature-induced plasticity in fitness-related traits may have differently evolved between habitats characterized by different host distribution in a parasitic wasp, as this environmental factor is expected to be crucial in the evolution of parasitoid life histories. Our main results are that (1) genetic differentiation occurs between habitats over small geographical distances; (2) habitat of origin –that significantly differed in host abundance, variance and distribution- explained differences in thermal reaction norms of several life history traits and in the level of phenotypic plasticity; and (3) the maximal investment in reproduction occurred at the lower temperature in orchards and the higher temperature in forests, independently of the climate of origin.

Genetic differentiation

We first observed that populations from orchards and forests, less than 10 km from each other, were genetically distinct in both climates. Such differentiation between populations suggests that they have evolved separately in different habitats and may thus be locally adapted. This result differs from a study on *Drosophila persimilis* (Taylor & Powell 1976) in which authors concluded that habitat differences in allozyme frequencies

over very short distances (less than 200m) were partly due to behavioral differences (i.e. habitat preferences) among genotypes.

Genetic differentiation over small distances has been rarely investigated in parasitoids but our result is consistent with the one observed between populations of the parasitic wasp *Diaeretiella rapae* less than 1km from each other in agricultural systems (Vaughn & Antolin 1998). Authors explained their results by the use of different hosts found in cultivated cereal or crucifer vegetable crops that were compared. To our knowledge, in Europe, *Leptopilina boulardi* only parasitizes *Drosophila melanogaster* and *D. simulans* which were both found in orchards and forests, *D. melanogaster* being largely dominant. It is thus unlikely that genetic differentiation in *L. boulardi* can be explained by differences in host species, but several factors can favour it. First, haplodiploidy, as found in *L. boulardi*, is known to result in a more pronounced genetic structure of populations in comparison to diploidy (Anton *et al.* 2007, Packer & Owen 2001). Secondly, this population subdivision may be favoured by fidelity to the habitat implying limited movements between forests and orchards (Anton et al. 2007). This is consistent with the dispersal ability observed in the field for other parasitoid species such as *Asobara tabida* (Ellers *et al.* 1998), another *Drosophila* parasitoid, that generally fly less than one kilometre. Our result suggests that the expansion of *Leptopilina boulardi* from the south to the north of France observed these last years (Fleury et al. 2004) may be more passive than active, for example thanks to the commercial fruits transports produced in the south, as described in Iran (Seyahooei *et al*. 2011).

Reaction norm patterns

We observed strong differences in reaction norms between females from forests and orchards under both climates. Females from orchards invested more in reproduction (i.e. had more eggs for a given body size, figure 1) at 20°C than 25°C while the opposite pattern was found for females from forests. It is unlikely that this difference in reaction norm is the result of evolution in response to microclimate because temperature is higher in orchards. A shift of the reaction norm toward higher temperatures should thus be observed there if plasticity evolved in response to this factor. At lower temperature, there is a physiological increase in lifespan in ectotherms as metabolic rate decreases and individuals use resources more slowly than at lower temperature (Brown 2004). An explanation for the observed pattern may thus be that this increase in longevity may lead to an inversion in the source of limitation of female wasps in orchards. Females from orchards may be time-limited at 25°C because of a short lifespan and should thus invest some resources in lifespan to lay all their eggs. At 20°C, females have a long lifespan because of the low temperature and they could find many laying opportunities over very short distances in orchards. They may thus be egg-limited at this temperature i.e. females have more laying opportunities than eggs, and a shift of resources to egg load may be adaptative in this habitat to prevent egg-limitation.

In insects, flight activity is known to increase with temperature until an optimum (e.g. Cox *et. al.* 2007, Williams & Osman 1960)). Forsse *et al.* (1992) have for example observed that, in the egg parasitoid *Trichogramma minutum*, between 70–80% of the wasps flew at 25 and 30 °C while less than 4% flew at 20 °C. In forests, females have to fly over long distances to find host patches, much farther than orchards parasitoids that can walk to find drosophila larvae. As temperature may increase their probabilities to find hosts, an increase

in investment of resources into reproduction may thus be adaptive at higher temperatures. That would explain that forests females invested more in reproduction, had a lower lipid content, and lived shorter than orchards females at the higher temperature, as a trade-of generally occurs between fecundity and the latter two traits (de Jong & van Noordwijk 1992, Ellers 1996, Rivero & Casas 1999, Ellers & van Alphen 1997).

Testing for more temperatures may be useful to clearly understand patterns of thermal reaction norms, as we measured these traits on females reared at only two temperatures. However, the thermal range of *L. boulardi* is really narrow in comparison to other insects, diapause occurring under 20°C and no development being observed above 28°C in our populations. More populations from both habitats should also be sampled in temperate, Mediterranean and other climatic areas to confirm out results on the effect of host distribution on the evolution of thermal reaction norm in parasitoids.

Level of phenotypic plasticity

Populations exhibited different levels of sensitivity to temperature for egg load, lipid content and longevity, depending on the habitat of origin. These results suggest relative canalization i.e. a decrease in the magnitude of plasticity for these three traits. Theoretical models predict that canalization in fitness traits should occur in individuals from variable environments (Stearns & Kawecki 1994; Wagner *et al*. 1997). However, even if any environmental factor may vary geographically and select for canalization, there are only evidences for thermal canalization of traits in response to climate of origin (Stearns *et al.* 1995, Liefting *et al.* 2009, Gilchrist & Huey 2004). This is quite new to observe variations in the level of thermal plasticity according to the habitat structure of origin.

We observed that the slope of egg load thermal reaction norm, the main component of fitness in parasitoids, was flatter for populations from forests, whereas the slope of longevity and lipid content was flatter for populations from orchards. Temperature is expected to be more variable in orchards than in forests; it is thus unlikely that differences in microclimate explain the decrease in the level of plasticity occurring in forests for egg load. Our field data suggest that forests represent a variable environment for laying opportunities; therefore, according to theoretical predictions, canalization should evolve for egg load in this habitat, as observed in our study. Egg load would thus be buffered against environmental variation and maximised in every condition. The opposite pattern for lifespan and lipid content suggests another environmental factor - variable in orchards and stable in forests - to be responsible for these traits. Temperature is more variable in orchards and may have selected for canalization in this habitat, but we should probably also observe differences between populations from the temperate and Mediterranean areas if this environmental factor was involved, as thermal amplitude is highly different between the two climates. Another explanation may be that lipid content and longevity are fitness traits but also physiological traits. Increased plasticity is known to be selected under variable environments for morphological and physiological traits (Bradshaw 1965, de Jong 1995). Thus, increased plasticity for longevity and lifespan as physiological traits may have been selected in forests in response to the variability of host distribution. A third hypothesis is that relative canalization in one trait, egg load in this study, may be traded-off with the strength of plasticity in other traits such as longevity or lipid content.

Conclusion

Parasitic wasps from forests strongly differed from orchards parasitoids for thermal reaction norms and level of phenotypic plasticity. It is likely that parasitoid populations are locally adapted to the host distribution, and not climate, though a larger sample size would be needed to confirm this result. This result is different from studies on *Drosophila*, in which thermal reaction norms are expected to have evolved in response to climate. This difference between a parasitoid and its host is probably the consequence of the direct link that exists in parasitoids between the number of hosts encountered and their number of progeny. Our study suggests that biotic factors varying with climate should be integrated in clinal studies comparing populations originating from different climates.

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	France Forest	France Orchard	Spain Forest	Spain Orchard
Mean temperature	17.1		21.0	
Thermal amplitude	20.1		13.1	
Nb of fruit flies found/trap	39 ± 39	268 ± 21	66 ± 49	190 ± 25
Inter-patch distance (m)	3.95 ± 2.3	1.95 ± 0.2	4 ± 2.4	2.2 ± 0.2
Nb of fruit flies emerging/trap	9 ± 12	74 ± 24	13 ± 12	68 ± 25
Nb of parasitoids emerging/trap	17 ± 11	122 ± 21	22 ± 13	116 ± 17

Table 1. Climatic data recorded for Vernet-les-Bains, close to Casteil (France) and La Bisbal d'Empordà, close to Calonge (Spain), from 2003 to 2007 (Source: MeteoFrance & Gencat). We considered average of each parameter measured every day from April to October, which is the period of activity of *Leptopilina boulardi*. Thermal amplitude was calculated as the mean of the differences between maximum and minimum air temperature per month.

Field data recorded in orchards and forests sampled in France and Spain in 2007 are also provided. Number of fruit flies/trap and distance to the closest patch were measured in the field and *Drosophila* and parasitoid emerging were counted in the lab. Error bars: ± s.e.

Life history trait	Factors	z/t value	standard error	p
Egg Load	habitat	4,488	0,662	7,19.10-6
	dry mass	-1,81	0,973	0,020
	temperature	2,728	0,015	0,006
	climatic area	1,148	0,546	0,251
	habitat x temperature	-4,736	0,028	2,18.10-6
Egg volume	habitat	-0,353	3,620	0,725
	dry mass	0,682	3,914	0,496
	temperature	0,513	1,524	0,609
	climatic area	0,811	2,921	0,419
	habitat x temperature	-0,878	1,700	0,381
Lipid content	habitat	-3,113	0,020	0,002
	dry mass	2,799	0,008	0,006
	temperature	-5,682	0,006	6,61.10-8
	climatic area	-1,01	0,188	0,314
	habitat x temperature	-2,433	0,042	0,016
Longevity	habitat	2,702	2,489	2,1.10-11
	dry mass	2,011	4,335	0,390
	temperature	4,874	1,784	0,014
	climatic area	1,234	1,856	0,220
	habitat x temperature	-6.9327	0,117	4.1e-9

Table 2. Results of the GLMs with the effects of habitat, climate of origin and rearing temperature on egg load (Poisson distribution), egg volume and lipid content (normal distribution) with dry mass as a covariate, and the corresponding Cox model for longevity.

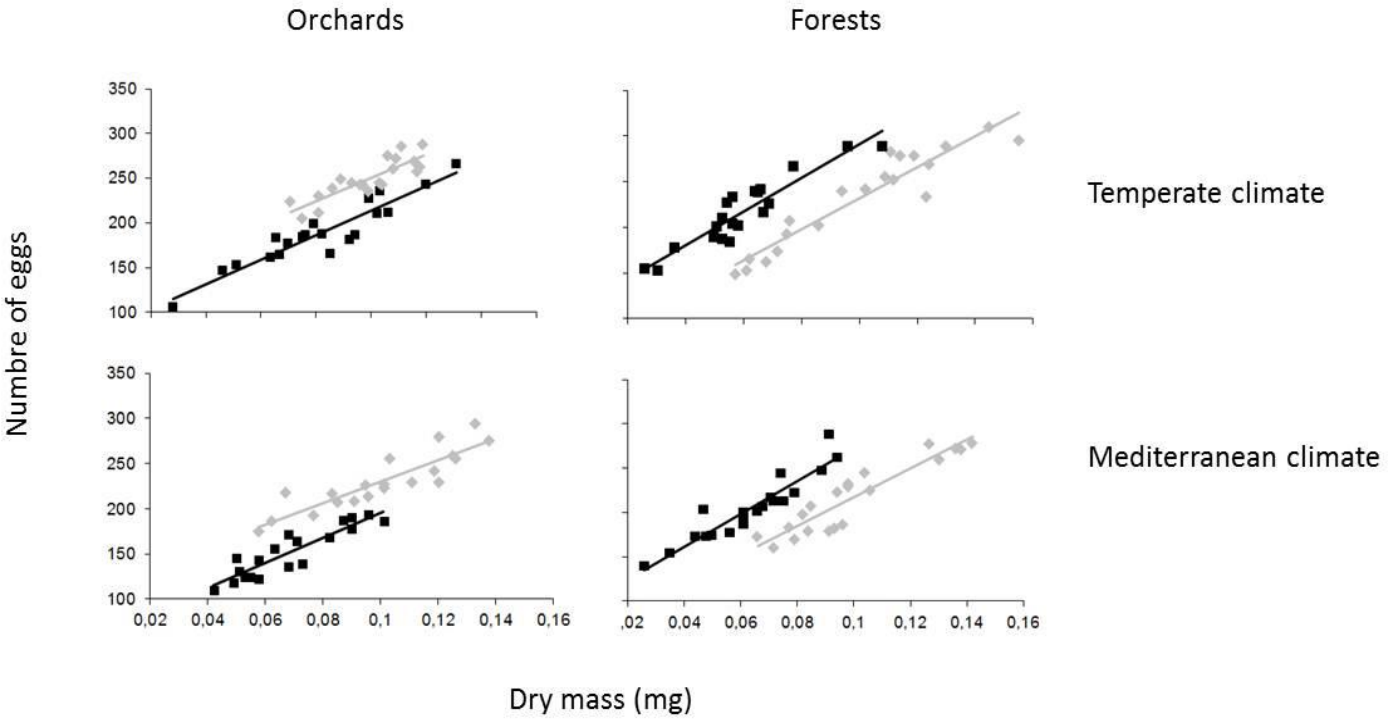

Figure 1. Plot of egg load at emergence according to the dry mass for the four populations reared at 20°C (grey diamonds) and 25°C (black squares).

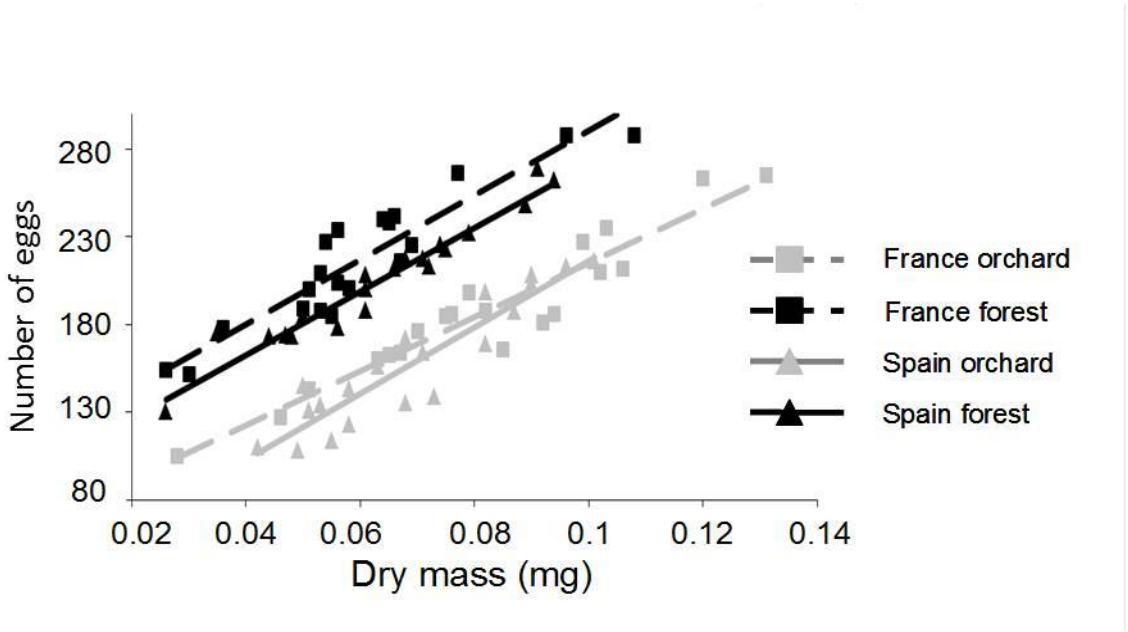

Figure 2. Plot of egg load at emergence according to the dry mass for the four populations reared at 25°C.

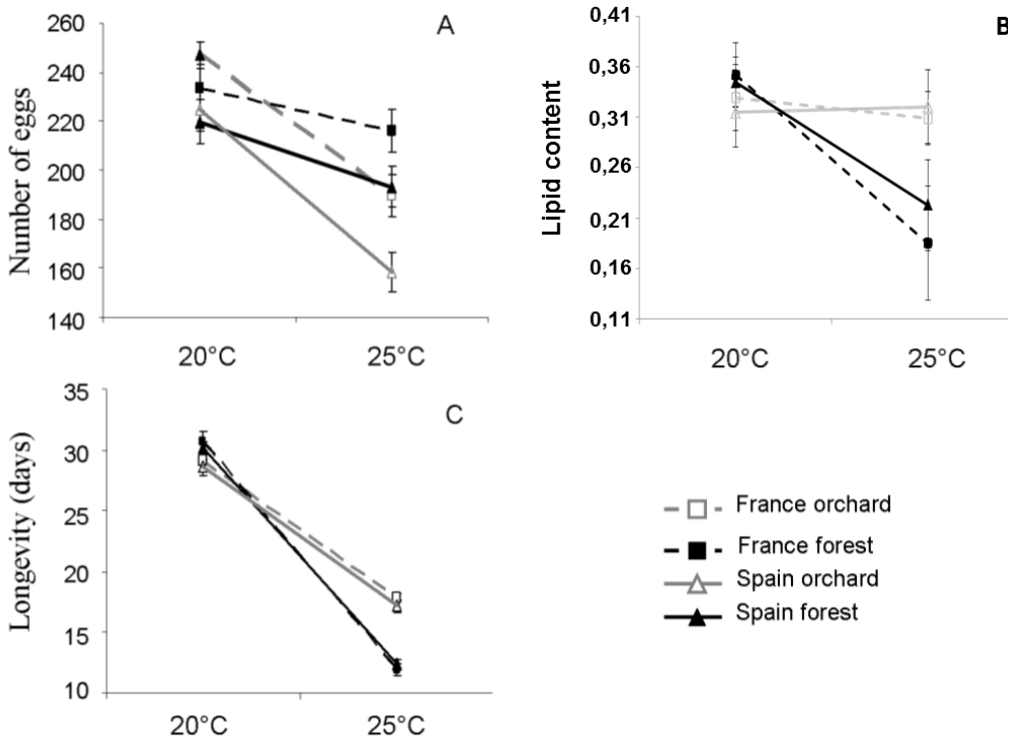

Figure 3. Mean ± s.e number of eggs (A), lipid content (B) and longevity (C) reaction norms across two temperatures.