Integrating large-scale geographic patterns in flight morphology, flight characteristics and sexual selection in a range-expanding damselfly

Lieven Therry, Hajnalka Anna Gyulavári, Sharon Schillewaert, Dries Bonte and Robby Stoks

While geographic trait variation along environmental clines is widespread, associated patterns in sexual selection remain largely unexplored. Geographic patterns in sexual selection may be expected if 1) phenotypes vary geographically and sexual selection is dependent on the local phenotypes in the population, and if 2) sexual selection is influenced by geographically structured environmental conditions. We quantified geographic variation in flight-related traits and flight performance in mated and unmated males and tested for geographic variation in sexual selection on these traits in the poleward range-expanding damselfly Coenagrion scitulum across a set of eleven core and edge populations ordered along thermal gradients in the larval and in the adult stage. We found little support for trait differentiation between core and edge populations, instead we found considerable geographic trait variation along the larval and adult thermal gradients. As expected under time constraints, body mass decreased with shorter larval growth seasons. Lower temperatures during the adult flight period were associated with a higher body mass, a higher flight speed and a higher fat content; these traits likely evolved to buffer flight ability at suboptimal temperatures and to optimize starvation resistance. Across the large geographic scale, we found a consistent higher flight duration in mated males. Instead, sexual selection for higher fat content was stronger in populations with lower adult flight temperatures and sexual selection for lower body mass acted only in edge populations. Our results indicate sexual selection on flight performance to be consistent over a large geographic scale and this despite the clear geographic patterns in sexual selection on the underlying morphological traits. Our results highlight that to fully understand the fitness implications of geographically changing trait patterns, researchers should consider the entire phenotype–performance–fitness axis and incorporate effects of geographically structured life-stage specific environmental conditions on this axis.
for larval growth and development. We expect smaller adults in populations with shorter growth seasons as animals are unable to fully compensate shorter growth seasons by faster growth (Blankenhorn and Demont 2004, Hassall 2013). Moreover, any compensatory acceleration in development rate can be traded off with investment in other traits (Stoks et al. 2006). The second thermal axis represents the thermal regime during the adult flight period. We expect a larger adult size in populations with lower adult flight period temperatures to optimize starvation resistance (Arnett and Gotelli 1999) and to reduce heat loss (Heinrich 1986). In populations situated at the expansion front we expect an increase in dispersal ability (Travis and Dytham 2002, Shine et al. 2011) and a higher investment in flight-related morphology (Hill et al. 2011).

Together with the study of geographic patterns in phenotypic traits, we studied whether sexual selection on these traits showed geographic patterns. We specifically tested three contrasting hypotheses: 1) sexual selection patterns are driven by geographic variation in the phenotypic traits as selection on a trait depends on the mean phenotypic trait value and its variation present in the population (Conner and Hartl 2004, Steele et al. 2011). 2) Sexual selection is directly driven by geographically structured environmental conditions, as these can modify the covariation pattern between a given trait and mating success (Moya-Laraño et al. 2007, Twiss et al. 2007), irrespective of any geographic patterns in the phenotype. 3) Sexual selection is not driven by geographic patterns in phenotypic traits nor by geographically structured environmental conditions (Blankenhorn et al. 2003).

As phenotypic traits we focused on flight-related morphology (fat content, flight muscle mass and body mass) and flight characteristics (flight duration and flight speed), which allowed direct testing for phenotype–performance relations. Given that males of scrambling damselflies are assumed to be under much stronger sexual selection than females (Corbet 1999) we only studied males. No differences in dispersal rates are apparent between sexes in Coenagrion species (Conrad et al. 2002), hence we have no reason to expect different evolutionary responses in flight ability during range expansion between males and females. We investigated sexual selection on these traits by comparing field-collected mated and unmated males, a well-established method to study sexual selection (Blankenhorn et al. 2003, 2004, Gosden and Svensson 2008). Phenotypic differences between mated and unmated males may indicate which traits are important to acquire mates, hence are under sexual selection. Yet, it should be taken into account that mating may also induce costs and therefore may cause differences in plastic traits such as energy reserves (Blankenhorn et al. 2003, 2004). Importantly, females are thought not to select males with a certain phenotype in scrambling damselflies (Fincke 1982), instead sexual selection for certain males occurs strictly through effects on their scrambling ability (hence flight ability). The chosen multivariate approach allows testing for geographic patterns in flight characteristics and the underlying traits, whether these patterns are consistent across traits and whether all traits show the same of the three predicted geographic patterns in sexual selection.

Material and methods

Study species and collection

The Mediterranean damselfly Coenagrion scitulum prefers small ponds (Dijkstra 2006). The species has an adult lifespan of ca 7–10 d (Angelbert and Giani 2003). Up to the 1990s the northern range limit was in northern France, where after a north-eastward range expansion occurred (Swagers et al. 2013). In 2010 and 2011, the most northern limit of the expansion front was situated in the north-west of the Netherlands, and the northeastern limit in western Germany and in the south of the Netherlands. During 2010 and 2011 we studied five core populations (C1–5) in France within the historical distribution of the species, and six edge populations (E1–6) in Germany, the Netherlands and Belgium; situated at the expansion front and founded less than five years before sampling. Three core populations (C1, C2 and C5) and one edge population (E6) were sampled in both years. The locations of the sampled populations and the entire species distribution are shown in Fig. 1.

In total 916 males (438 unmated, 478 mated) were collected between 25 May and 24 July in 2010 and 2011 (for details see Table 1). Mated males are defined as males that were observed together with a female in tandem position, copulation or oviposition (Thompson et al. 2011), while unmated males were single males searching for females at the breeding pond. We sampled only males that 1) were active at the reproduction site during sunny days and at moments when sexual activity was observed, and 2) showed no visible wing damage. Note this also standardized the age across mated and unmated males. The univoltine life-cycle of the species (Cayrou and Céréghino 2005) avoids complications due to changing generations over the season (cf. Hassall 2013). In order to estimate the local competitive environment which may influence sexual selection (Punzalan et al. 2010) we scored population densities and sex ratios when activity levels of C. scitulum were at their daily peak (Supplementary material Appendix A). Due to time constraints we were unable to subject all collected males to the flight performance test. In total 644 males (313 unmated, 331 mated, Table 1) were included in the flight tests. In the first sampled population in 2010 (population C1), flight duration and not flight speed was measured.

Quantification of flight characteristics and flight morphology

Two components of animal flight, flight speed and flight duration, were scored in situ (for details see Supplementary material Appendix B). Afterwards, each male was flash-frozen in liquid nitrogen (−196°C) and stored until the quantification of flight-related traits. Three flight-related morphological traits were quantified based on protocols described in Swillen et al. (2009): fat content, flight muscle mass and dry body mass (for details see Supplementary material Appendix B). Fat content is the largest source of energy reserves in insects (Kaufmann et al. 2013) and the flight muscle mass is a good predictor of power output in Odonata (Schilder and Marden 2004).
Estimating population-specific larval and adult thermal regimes

To test for an effect of the geographically structured thermal regimes on flight characteristics and underlying morphological traits, we separately reconstructed for each population the thermal regime for the aquatic larval stage where growth occurs and the adult terrestrial stage where reproduction occurs (Supplementary material Appendix C).

Statistical analyses

Data were analyzed using mixed models (SAS ver. 9.3) with the replicated populations nested in the range status (core versus edge) as random effects. Significance of random factors was verified with likelihood ratio tests (Littell et al. 1996). To simplify final models, non-significant interactions were step-wise removed using backward selection.

We analyzed the effects of range status, mating status (mated vs unmated), both thermal regimes (larval degree days and adult flight period temperature) and year on the response variables with AN(C)OVAs. Body temperature was added as covariate when testing flight duration and flight speed to take into account the temperature-dependence of flight performance (Samejima and Tsubaki 2010). The length of the time interval between the collection of the males and the start of the flight test was added as a covariate in the analysis of flight speed. Body mass was

Table 1. Total sample sizes and dates of sampling for unmated and mated males and sample sizes for the subset of males for which flight performance variables were quantified for each population of C. scitulum sampled in 2010 and 2011. Population codes starting with C (E) refer to core (edge) populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>Code</th>
<th>Year</th>
<th>Sample dates</th>
<th>Total sample size</th>
<th>Sample size flight performance</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>unmated mated</td>
<td>unmated mated</td>
</tr>
<tr>
<td>Le Bouchet</td>
<td>C1</td>
<td>2010</td>
<td>5–7 June</td>
<td>22 37</td>
<td>11 10</td>
</tr>
<tr>
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<td>C2</td>
<td>2010</td>
<td>16–18 July</td>
<td>9 5</td>
<td>8 5</td>
</tr>
<tr>
<td>Vauville</td>
<td>C3</td>
<td>2010</td>
<td>21–22 June</td>
<td>27 24</td>
<td>15 12</td>
</tr>
<tr>
<td>Roussent</td>
<td>C4</td>
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<td>18–24 June</td>
<td>39 28</td>
<td>31 19</td>
</tr>
<tr>
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<td>C5</td>
<td>2010</td>
<td>25–28 June</td>
<td>14 18</td>
<td>12 18</td>
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<tr>
<td>Zülpich</td>
<td>E2</td>
<td>2010</td>
<td>5–8 July</td>
<td>31 31</td>
<td>22 25</td>
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<tr>
<td>Knokke</td>
<td>E4</td>
<td>2010</td>
<td>30 June–1 July</td>
<td>33 32</td>
<td>25 27</td>
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<td>Cadzand</td>
<td>E5</td>
<td>2010</td>
<td>2–3 July, 10–14 July, 20–22 July</td>
<td>46 48</td>
<td>22 25</td>
</tr>
<tr>
<td>Hoofdplaat</td>
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<td>14–15 July, 24 July</td>
<td>24 22</td>
<td>12 10</td>
</tr>
<tr>
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<td>42 44</td>
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<tr>
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<td>4–7 July</td>
<td>4 17</td>
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<tr>
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<td>24–27 June</td>
<td>28 38</td>
<td>25 22</td>
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<tr>
<td>Blieskastel</td>
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<td>2011</td>
<td>11–15 June</td>
<td>24 32</td>
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<td>Cottessen</td>
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<td>17–21 June, 11–12 July</td>
<td>26 34</td>
<td>21 22</td>
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<tr>
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<td>E6</td>
<td>2011</td>
<td>25 May, 29 June–1 July</td>
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<td>51 62</td>
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Total sample size 438 478 311 331
added as covariate when analyzing fat content and flight muscle mass. In these models, interactions between mating status and range status or between mating status and the thermal regimes indicate geographic patterns in sexual selection. To specifically correct for possible confounding effects of sampling date, local population density and sex ratio on trait values and sexual selection regimes, we added Julian day, log-transformed population densities and log-transformed sex ratios estimated at the sampling day to all statistical analyses. As these ANCOVAs only allow direct testing for directional sexual selection on the response variables (which would be indicated by an effect of mating status), we additionally investigated the presence of quadratic selection on all variables (Supplementary material Appendix D). Data on the covariation between the phenotypic traits and the flight characteristics are reported and discussed in Supplementary material Appendix E.

### Results

There was no geographic signal in flight duration: it did not differ between core and edge populations, and was not affected by the thermal regimes (Fig. 2a, Table 2). Flight speed also did not differ between core and edge populations (Fig. 2b, Table 2), yet it decreased in populations with higher adult flight period temperatures (slope $\pm$ 1 SE: $-0.062 \pm 0.021$, Table 2, Fig. 3a). Mated males did not differ in flight speed compared to unmated males but consistently showed longer flights (Table 2) indicating sexual selection for longer flight duration. Males had longer and faster flights in 2011 than in 2010 (Table 2). Males with higher thorax temperatures flew longer (slope $\pm$ 1 SE: $0.0277 \pm 0.0074$) and at higher speeds (slope $\pm$ 1 SE: $0.0325 \pm 0.0041$, Table 2).

Fat content did not differ between core and edge populations (Table 2, Fig. 4a), yet was lower in populations with higher adult flight period temperatures (slope $\pm$ SE: $-0.077 \pm 0.024$, Table 2, Fig. 5b). Furthermore, variance in fat content was lower in populations with higher adult flight period temperatures ($F_{1,12} = 9.03$, $p = 0.0110$, slope $\pm$ SE: $-0.034 \pm 0.011$, Fig. 5c). Overall, mated males had a higher fat content than unmated males (Mating status, Table 2). Moreover, this effect depended upon the adult flight period temperature ($AFPT \times$ Mating status, Table 2) reflecting a pattern whereby sexual selection on fat content was higher in populations with lower adult flight period temperatures (Fig. 5a). Fat content was higher in 2011 than in 2010 (Table 2).

Overall, flight muscle mass and body mass did not differ between core and edge populations. Body mass increased in populations with more larval degree days (slope $\pm$ 1 SE: $0.0055 \pm 0.0013$, Table 2, Fig. 3b) and decreased in populations with higher temperatures during the adult flight period (slope $\pm$ 1 SE: $-0.57 \pm 0.12$, Table 2, Fig. 3c). Overall, flight muscle mass and body mass did not differ between mated and unmated males (Table 2, Fig. 4b–c). Differences in body mass between mated and unmated males were dependent on the combination of range status and year (Range status $\times$ Mating status $\times$ Year, Table 2), with mated males having a nearly significant lower body mass than unmated males only in edge populations in 2010 ($F_{1,249} = 2.93$, $p = 0.088$). Males had a higher flight muscle mass and a lower body mass in 2011, although the latter was only the case for edge males (Range status $\times$ Year, Table 2).

### Discussion

**Geographic patterns in trait differentiation**

We found little support for trait differentiation between edge and core populations. This is opposite to general expectation (Hill et al. 2011) and to the results from a common garden rearing experiment where *C. scitulum* from edge populations had a higher relative flight muscle mass compared with those from core populations (Therry et al. 2014) which was likely shaped by evolution of higher dispersal rates. We see two reasons why we did not observe the differentiation detected under common garden conditions between core and edge populations in current field study. First, there was quite some variability in fat content,
Table 2. AN(C)OVAs testing for the effects of range status (RS: edge vs core), mating status (Mat: mated vs unmated), year (2010 vs 2011) and the larval (LDD) and adult (AFPT) thermal regimes on the flight characteristics (flight duration, flight speed) and the morphological traits (fat content, flight muscle mass and body mass) in *C. scitulum* males. Flight performance traits were corrected for thorax temperature; fat content and muscle mass were corrected for body mass; flight speed was corrected for the time kept in the cooling box (Time in box). All analyses are corrected for Julian day, density and sex ratio at the day of collection. Significance values are indicated as *p* < 0.05, **p** < 0.01, ***p*** < 0.001.

<table>
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<th>Flight muscle mass</th>
<th>Body mass</th>
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<td>F</td>
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<td>year</td>
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<td>1,944</td>
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<tr>
<td>Mat × year</td>
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<tr>
<td>RS × Mat × Year</td>
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<td>1,609</td>
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<td>Time in box</td>
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<td>Body mass</td>
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<td>Julian day</td>
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<tr>
<td>AFPT</td>
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Random effect

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<td>28.8***</td>
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<td>52.7***</td>
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Figure 3. Geographic covariation patterns between (a) flight speed and adult flight period temperature, (b) body mass and the number of larval degree days, and (c) body mass and adult flight period temperature. Symbols represent means (± 1 SE) of population-by-year combinations. For flight speed the mean residuals obtained from the model containing range status, year, mating status and body mass; and for body mass the mean residuals obtained from the model containing range status, year and mating status are shown.
flight muscle mass and body mass among populations of the same range status (Table 2), possibly due to differences in unmeasured local conditions such as food availability (Nylin and Gotthard 1998, Stoks and Cordoba-Aguilar 2012). Second, although we found no overall difference in temperature regimes (Supplementary material Appendix C), population densities or sex ratios between core and edge populations (Supplementary material Appendix A), suboptimal conditions that are assumed to prevail at the edge of species' ranges (Hardie and Hutchings 2010) may have also masked phenotypic differentiation between core and edge populations.

Instead, we found considerable support for geographic variation in traits associated with the larval and adult thermal regimes. Individuals from populations with shorter larval growth seasons had a lower body mass, reflecting time constraints for larval development (Blanckenhorn and Demont 2004). This pattern in body size is predicted to occur especially in univoltine species (Johansson 2003) such as *C. scitulum*. Under time constraints, damselfly larvae typically increase development rate but not necessarily growth rate, resulting in a smaller mass in the adult stage (De Block and Stoks 2004) as explicitly shown in other coenagrionid damselflies (Strobbe and Stoks 2004, Sniegula et al. 2012). Since we measured body mass on field-collected animals we are unable to distinguish between plastic and genetic contributions to the observed clinal trait patterns. Moreover, we cannot exclude the possibility that the clinal trait patterns are indirectly driven by other factors covarying with the thermal regimes, such as food availability (Arnett and Gotelli 1999). We, however, can exclude an effect of conspecific competition driven by changes in population densities as these did not covary with either of the population-specific thermal regimes (Supplementary material Appendix A). The finding that the here studied thermal regimes covary with different traits, such as development time, growth rate and flight muscle mass in *C. scitulum* reared under common garden conditions (Therry et al. 2014) suggests that the observed geographical patterns in trait variation are at least partly genetically determined and driven by the thermal regimes per se.

Given that Odonata are flying ectotherms and only active when their body temperature is high enough (Samejima and Tsubaki 2010), it is not surprising that also the adult thermal regime affected flight-related traits. The observed higher flight speed (corrected for body temperature) in populations with lower adult flight period temperatures likely reflects a compensation for a reduced flight muscle efficiency at lower temperatures (Hassall et al. 2008). The presence of larger males in populations with lower temperatures during the adult flight period can be explained by the fact that larger males have 1) a lower temperature loss through convective cooling (Heinrich 1986) and 2) a better ability to withstand starvation (Arnett and Gotelli 1999). The latter

Figure 4. Mean (+ SE) fat content (a), flight muscle mass (b) and body mass (c) in unmated and mated males from core and edge populations of *C. scitulum* in 2010 and 2011. Shown are least-square means. Fat content and flight muscle mass are corrected for body mass.
mechanism probably also explains the finding of higher fat contents in populations with lower adult flight period temperatures.

**Sexual selection and its geographic variation**

In scramble competitors, such as *C. scitulum*, the outcome of competition among males is expected to be mainly determined by the ability to perform active searching flights (Andersson 1994). Flight performance is therefore an important selection target in scrambling species, yet rarely directly measured in sexual selection studies (Husak and Fox 2008, Kelly et al. 2008). Because males *C. scitulum* avoid resting on horizontal surfaces (such as the bottom of the flight tube), we expect that males were highly motivated to fly, and that the flight duration reflects flight endurance. However, we cannot exclude that a behavioural component was included linked to general activity and exploratory behaviour. This, however, would not invalidate the relevance of our performance measure which in case of an influence of these behavioural components would reflect ecological performance (sensu Irschick 2003) rather than maximum performance. Ecological performance is often lower than maximum performance due to a behavioural component (Irschick 2003), which is especially expected if maximal performance is not required for accomplishing an ecologically relevant task (Husak 2006). For example, maximum sprinting speed determines predation risk in hatchlings of the collared lizard. In contrast, the faster adults can attain a speed necessary to escape predators by using less of their maximal capacity and predation risk is determined by behavioural flee decisions, thereby partly decoupling actual escape speed and maximal sprinting speed (Husak 2006). This suggests that ecological performance may be more important for selection than maximum performance (Husak 2006, Careau and Garland 2012). We detected positive directional sexual selection for a longer flight duration. We did not detect sexual selection for higher flight speeds which indicates that endurance is likely more important than maximum speed in scramble competitors (as suggested by Husak and Fox 2008). Importantly, the selection pattern on flight duration was consistent across edge and core populations and along the thermal regimes which parallels the observation that flight duration itself did not show a geographic signal.

In accordance with their covariation pattern with flight duration, we found sexual selection for a higher fat content and for a lower body mass. For flight duration and fat content we can unambiguously interpret the higher values in mated males as a result of sexual selection through scrambling competition. Although we cannot fully exclude the
possibility that the smaller body mass of paired males is due to mating costs, this seems unlikely given that we did not find a cost signal in fat content, the main storage pool in insects (Kaufmann et al. 2013). Moreover, the mass pattern is to be expected given its negative covariation pattern with flight duration (Supplementary material Appendix E). In contrast to the geographic consistent sexual selection pattern for a high flight duration, selection patterns on fat content and body mass showed a strong geographical signal indicating that their covariation with mating success was not entirely via flight duration.

Sexual selection for higher fat levels (as shown before in a scrambling fly, Blanckenhorn et al. 2004) was more pronounced in populations with lower adult flight temperatures during the flight period. This pattern can be explained by two non-exclusive mechanisms. Firstly, this pattern may reflect a higher dependency of energy storage when scrambling in populations with less favorable thermal regimes during the flight period. In such populations mating efficiency is expected to depend more strongly on the ability to endure poor weather conditions and selection on traits such as fat reserves is expected to be stronger (Purse and Thompson 2005). Secondly, sexual selection may depend on the phenotypic distribution of the trait in the population (Conner and Hartl 2004) and the mean and variance in fat content were higher in populations with lower average temperatures during the flight season. For example, the higher phenotypic variance in fat content may have facilitated the ability to detect the occurrence of sexual selection in populations with lower temperatures.

In scrambling competitors, a small male advantage for mate acquisition is expected as small males require lower amounts of food, hence can spend more time searching for females (Blanckenhorn et al. 1995). We indeed detected sexual selection for a lower body mass, yet only in the edge populations in 2010. Temperatures during the flight period were higher in edge populations in 2010 (Supplementary material Appendix F), and this likely changed the covariation pattern between body mass and flight performance and thereby changed the sexual selection on this trait between the core and edge populations (Moya-Laraño et al. 2007).

**Conclusions**

Given that the edge-core differentiation and the studied thermal clines did not covary we could disentangle the effects of range status and thermal clines in shaping the studied geographic variation in flight-related traits. While we found no trait differences between the core and edge populations, there were clear geographic patterns in trait variation along the geographically structured larval and adult thermal regimes. Moreover, and in line with the complex life cycle with larvae and adults experiencing different selection pressures (Stoks and Cordoba-Aguilar 2012), larval and adult traits responded in opposite ways to the stage-specific thermal regimes.

A first striking finding was that sexual selection on flight duration, a measure of flight performance, was consistent at the large geographic scale, in contrast to the clear geographic patterns in sexual selection on two underlying morphological traits (fat content and body mass). This is consistent with the idea that sexual selection acts mainly on performance and only secondarily on the underlying traits (Husak and Fox 2008), whereby the translation of morphological traits to performance may depend on environmental conditions (Samejima and Tsubaki 2010). Another key finding was that geographic patterns in sexual selection on a given trait are not necessarily associated with a geographic pattern in that trait, which was the case for body mass. In contrast, sexual selection for higher fat content was associated with a pattern of higher means and variances of fat values in populations with lower temperatures during the flight period. The latter pattern may reflect a direct dependence of the selection pattern on the phenotypic distribution (Conner and Hartl 2004, Steele et al. 2011), and/or a physiological pattern where adult flight period temperatures modified the covariation pattern between fat content and mating success.

Taken together, the followed multivariate approach applied at a large geographic scale that focused on phenotype–performance–fitness covariation patterns allowed us to document the poorly studied (Blanckenhorn and Demont 2004) fitness consequences of geographic patterns in trait variation and also illustrated the trait-specificity of the associations between geographic patterns in trait values and sexual selection patterns. Our results highlight that to fully understand the fitness implications of geographically changing trait patterns, researchers should incorporate effects of geographically structured environmental conditions and should not only consider the phenotypic traits under selection but also the associated performance measures. Furthermore, given opposite covariation patterns between traits and stage-specific thermal regimes are expected and here documented between the larval and adult stage, stage-specific thermal regimes should be quantified when explaining trait variation along geographic clines. The here applied multivariate approach is a promising tool to shed light on the largely unknown fitness implications of well-known latitudinal gradients such as the Bergmann clines in body size (Blanckenhorn and Demont 2004) as it allows to tell apart the effects of geographically structured environmental conditions versus geographically structured trait distributions in shaping geographic patterns in sexual selection.

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**References**


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Supplementary material (Appendix ECOG-00630 at <www.ecography.org/readers/appendix>). Appendix A–F.