

Patterns of Phenotypic Divergence in Wing Covariance Structure of Calopterygid Damselflies

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Abstract Comparing species differences in covariance patterns of traits subject to divergent selection pressures can increase our understanding to the mechanisms of phenotypic divergence. Different species of calopterygid damselflies have diverged in the melanized wing patch of males. This trait serves multiple ecological functions and has behavioral consequences in terms of sexual selection, interspecific interactions, reproductive isolation. We compared the phenotypic variance-covariance matrices (\mathbf{P}) of wing traits among nine populations of four European species of calopterygid damselflies. We found modest divergence in covariance structure among populations of the same species, but strong divergence between species. Interestingly, the orientation of the first eigenvector of \mathbf{P} (\mathbf{P}_{\max}) differed more between closely related species than between distantly related species, although this pattern was absent when overall covariance structures were compared. We also found that distantly related species but geographically closer had converged towards a similar covariance structure. Finally, divergence in covariance structure was correlated with divergence in wing patch length, but not with other wing traits. This last finding suggests that divergent selection on wing patch length might have affected the stability of \mathbf{P} . These results indicate that \mathbf{P} might not only reflect ancestral developmental pathways but might also be influenced by current ecology.

Keywords Phenotypic integration · Phenotypic plasticity · P-matrix · Speciation · Wing morphology

Introduction

Organisms are integrated systems that have to be jointly mutable and adaptable to the constantly changing environment. However, organisms must at the same time also be sufficiently stable so that they preserve their functionality through development (Debat and David 2001; Willmore et al. 2007; Nijhout 2008) and functionality must also be buffered against environmental perturbations (Wagner and Altenberg 1996; Polly 2008). Divergence along genetic or phenotypic “lines of least resistance” has been reported for a variety of phenotypic traits in different species (Schluter 1996, 2000), suggesting that covariance structures might reflect constraints on phenotypic evolution (Arnold 1992). In ecological speciation research, researchers typically focus on ecologically relevant traits that also have important functions in reproductive isolation (Schluter 2000). If traits mediating adaptation to alternative environments are genetically correlated with traits conferring reproductive isolation, then speciation can emerge as a correlated response to divergent selection (Schluter 2000; Funk et al. 2006). A deeper understanding how such correlated phenotypic traits have jointly diverged among closely species of the same genus is likely to shed light on the mechanisms behind phenotypic evolution (Game and Caley 2006).

A hotly debated topic in evolutionary biology is whether the covariance structure of a set of traits mainly reflects the ancestral and shared developmental pathways underlying the traits, or to what extent, if at all, covariance structures reflect past selective pressures on the same set of traits (Cheverud 1984; Arnold et al. 2001; Wallace 2009). It has

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long been acknowledged that development biases can influence the direction of evolutionary trajectories (Maynard Smith et al. 1985; Wallace 2009). However, some workers argue that selection can break up such developmental biases and eventually re-shape the covariance structures at least under certain conditions (Beldade et al. 2002). The constancy, and instability, of the phenotypic and genetic covariance structures has been addressed extensively in the past decade (see Arnold et al. 2008 for review). Explaining why and how covariance structures evolve through time in adaptive radiations remains a challenging issue. The difficulties arise because it is logistically difficult to obtain large amounts of data to estimate patterns of covariation across different species or populations while at the same time also investigating the selective pressures which operates on the same species or populations.

In a recent study on the covariance structure of two species of calopterygid damselflies (*Calopteryx splendens* and *C. virgo*), we found both greater divergence among populations of different species than among populations of the same species and a concordance of within- and between-population correlations within one of these species (Eroukhmanoff and Svensson 2008). These observations indicate that the “line of least resistance” or the orientation of the first eigenvector is conserved across populations but not necessarily across species/taxa (Schluter 1996). These more pronounced differences between species could reflect different selective pressures, since after sufficient time under restrained gene flow, the covariance structure of a population is expected to conform to the shape of the adaptive landscape (Wallace 2009). Wing morphology and wing coloration in these species have been shown to be targets of both natural and sexual selection (Svensson et al. 2004; Tynkkynen et al. 2004; Svensson et al. 2006; Svensson and Friberg 2007) and at the multivariate level, divergence might result from correlational selection for different optimal wing character combinations (Svensson and Friberg 2007). Other recent studies on wing morphology in insects have demonstrated clinal variation in wing length, and these parallel latitudinal clines arise through an interaction between similar selection pressures in similar environments and historical contingencies on the different continents (Huey et al. 2000; Pelabon et al. 2006). Schluter (1996) argued that covariance structure might bias the direction of evolutionary change, particularly in the early stages of divergence. Comparing divergence in covariance structures of wing morphology at different taxonomic and geographic levels could therefore add to our general understanding of how covariance structure might reflect biases in the direction of evolutionary change at different stages of divergence (Eroukhmanoff and Svensson 2008).

Here, we present data from a comparative study on divergence in phenotypic variance–covariance matrices (**P**) data from nine populations of four species of calopterygid damselflies. We estimated and compared the magnitude of shared covariance structure in wing morphology between the different populations and species in this genus. The wing morphology of this damselfly genus is unusually well-investigated in terms of natural and sexual selection (Siva-Jothy 1999; Svensson et al. 2004; Córdoba-Aguilar and Cordero-Rivera 2005; Svensson et al. 2006), interspecific interactions (Tynkkynen et al. 2004; Svensson et al. 2007), sexual isolation (Svensson et al. 2007) and predator-mediated natural selection (Svensson and Friberg 2007). Results in this study indicate that although covariance structure is more conserved at the intraspecific level than at the interspecific level, the influence of local environmental factors and local ecologies is also important. We demonstrate that the amount of divergence in covariance structure is correlated with the amount of divergence in wing patch length, a trait that is target of several different (and sometimes opposing) selective pressures (see above). This confirms the idea that the phenotypic covariance structure of functionally important sets of traits does not only reflect shared ancestral developmental pathways but might also reflect current selective pressures and local ecology (Polly 2008; Wallace 2009; Jamniczky and Hallgrímsson 2009).

Melanin wing patches in males of the genus *Calopteryx* differ in size between different species and between populations within species, as does other wing morphological traits such as wing length and wing width (Eroukhmanoff and Svensson 2008). The signal efficiency of sexually selected trait like these melanin wing patches can potentially be increased by increasing the size of the melanin patch itself, independent of other aspects of wing morphology, and the total signal output will then ultimately be constrained by the total wing area covered by melanin. Alternatively, although certainly not mutually exclusive, wing length and wing width can be increased independently of wing melanization which in turn might provide further opportunity for future exaggeration of wing melanization. Recent field studies have demonstrated that avian predators impose correlational selection on these wing traits, so that wing patch size, wing length and wing width are selected in combination with each other and are thus unlikely to evolve as single traits in isolation (Svensson and Friberg 2007). Analyzing wing covariance structure thus is a natural step forward from our previous ecological studies and field work in these natural populations. The wings of *Calopteryx* and other odonate genera can be viewed as developmental modules (Wagner and Altenberg 1996), that are under selection to become integrated to functional units for multiple adaptive purposes (Wotton and Newman 2008). This is because the wing traits are simultaneously targets of natural

and sexual selection pressures in addition to wing melanization functioning as a species recognition character (Svensson et al. 2007). Finally, one of the most important functions of odonate wings is of course aerodynamic performance and its effects on flight efficiency (Usherwood and Lehmann 2008). Escape performance to avoid flying predator enemies should impose strong selection on wing length, wing width, and wing shape (Hedenstrom and Rosen 2001; Svensson and Friberg 2007).

Methods

Study Species

Calopteryx splendens, *C. xanthostoma*, *C. virgo meridionalis*, and *C. v. virgo* (Odonata: Zygoptera), are four demoiselle taxa that all occur in Europe (Askew 1988). *C. splendens* and *C. virgo virgo* both occur in northern and central Europe, including southern Sweden, whereas *C. xanthostoma* and *C. virgo meridionalis* are restricted to southern Europe, i.e., Spain and France (Askew 1988). These odonate species are similar in body morphology and behavior (Corbet 1999), except for the wing morphology of males: *C. splendens* and *C. xanthostoma* males have dark melanin-patches on both forewings and hindwings, covering 50–70% of the wing area and they are considered to be sister species (Corbet 1999; Dumont et al. 1993; Weekers et al. 2001). Two subspecies (*C. v. virgo* and *C. v. meridionalis*) have males with more than 90% of their wings covered with melanin (Askew 1988; Corbet 1999; Fig. 1a). Other aspects of male morphology are similar in all species: long and thin blue abdomen, blue-green thorax and large black eyes.

Data Collection and Wing Morphology

In southern Sweden, in the “Skåne” region, we measured a total of 449 *C. splendens* males in one population and 502 *C. splendens* males and 246 adult *C. virgo virgo* males in one sympatric population during 2002 and 2003 (Eroukhmanoff and Svensson 2008). In northern and central Spain, we measured a total of 20, 26, and 30 *C. xanthostoma* males and 29, 30, and 30 *C. virgo meridionalis* males in three different sympatric populations containing both of these southern species in 2006. We measured three different wing traits: wing length, wing width, and wing patch length (to the nearest 0.01 mm) using digital calipers. We used ANOVA with species as a categorical factor to test whether the traits differed between species and a nested ANOVA with population nested within species as categorical factors to test whether the traits also differed between populations.

Comparing Covariance Structures

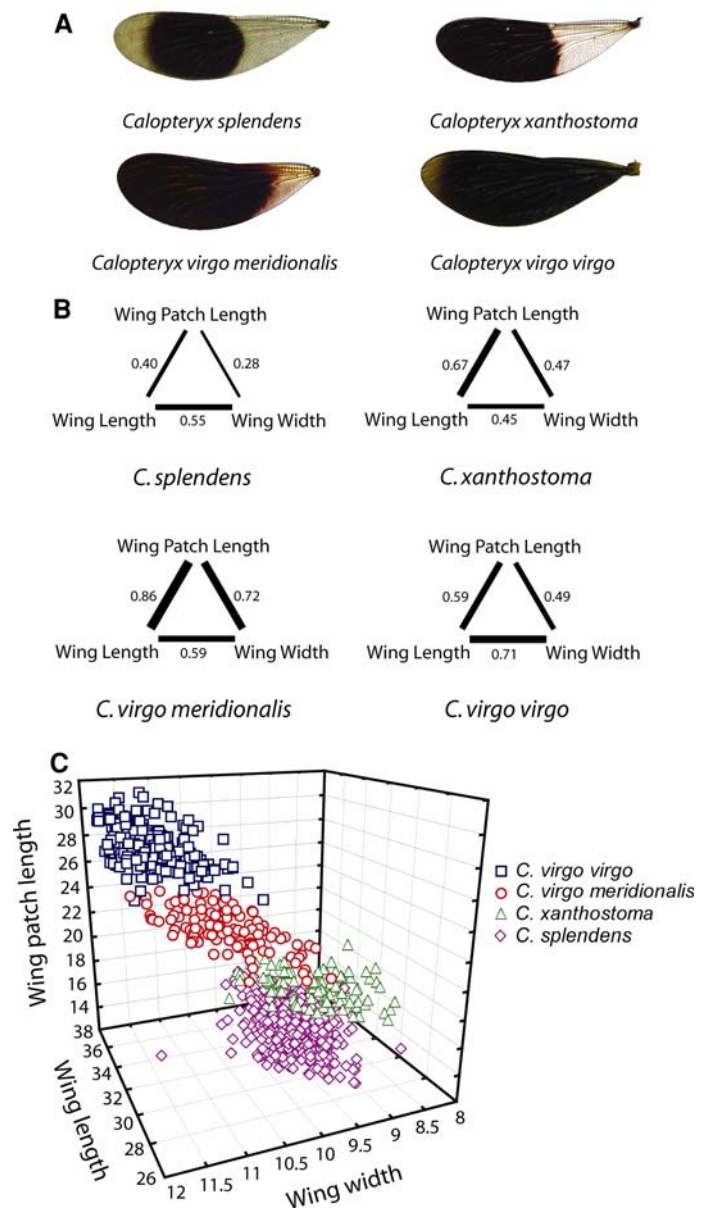
Phenotypic variance–covariance matrices were calculated for all species ($N = 4$) and populations ($N = 9$) using the software STATISTICA (Statsoft, Inc 2004). To assess P-matrix divergence between the different species and populations, we calculated θ , the angle between the \mathbf{P}_{\max} among different P-matrices. This procedure has been used to quantify the differences between the directions of the lines of maximum variance (Eroukhmanoff and Svensson 2008), the so-called phenotypic lines of least resistance or \mathbf{P}_{\max} (Schluter 1996).

In total, our analyses included seven pairwise comparisons between populations of the same species (*C. splendens*, *C. virgo meridionalis*, and *C. xanthostoma*), nine pairwise comparisons between populations of closely related species (*C. splendens* and *C. xanthostoma* or *C. v. meridionalis* and *C. v. virgo*), and 20 pairwise comparisons between populations of distantly related species (*C. xanthostoma* and *C. v. meridionalis* or *C. v. virgo*, or *C. splendens* and *C. v. meridionalis* or *C. v. virgo*). These three categories of comparisons are of course not statistically independent, since they involve the use of the same populations multiple times. To account for statistical non-independence between these different comparisons, we therefore, compared the differences between the categories with a permutation-based resampling procedure with 1,000 replications, using the software Resampling Stats (Simon 2000) as we did in a previous study involving *C. splendens* and *C. virgo* (Eroukhmanoff and Svensson 2008).

To investigate divergence in more detail in the distantly related species, we divided these comparisons into two categories: 11 comparisons involving species in close geographical proximity (*C. xanthostoma* and *C. v. meridionalis* or *C. splendens* and *C. v. virgo*) and nine comparisons involving species that were geographically more distant from each another (*C. xanthostoma* and *C. v. virgo* or *C. splendens* and *C. v. meridionalis*).

We also compared the overall covariance structure using the random skewers method (Cheverud 1996). This enabled to compare in a similar pairwise fashion how the different P-matrices could bias the response to random selection vectors. This technique computes the responses of each covariance matrix to a given number of random selection vectors, and then compares these responses to compare covariance structures. In the present analysis, we used the program “Skewers” (Revell 2007). We computed 10,000 random skewers for all possible pairwise comparisons. The responses can then be averaged and analyzed with an ANOVA to determine similarity in the overall covariance structure of the compared groups (Jamniczky and Hallgrímsson 2009). Finally, we also used the vector correlations to plot the amount of divergence in wing covariance structure with the

Fig. 1 Divergence in wing-patch length, a secondary sexual trait, between calopterygid species. **a.** Wing photographs of the four calopterygid species studied. *Calopteryx splendens* and *C. xanthostoma* are sister species, whereas *C. virgo meridionalis* and *C. v. virgo* are two subspecies of *C. virgo*. **b.** Phenotypic correlations between the three wing traits investigated. All correlations between traits are positive, but they differ in magnitude between traits and species. **c.** The wing morphospace involving the three traits measured in this study (wing width, wing length and wing patch length). The four species occupy different regions in overall morphospace, although they are mainly clustered along the wing patch length axis



amount of divergence in wing traits among the different pairwise comparisons. We used a Mantel test with 10,000 permutations to test for the significance of these correlations.

Results

Multivariate Divergence in Wing Morphology

Although the main striking difference between species is the melanized wing patch ($F_{3,920} = 5883.4, P < 0.0001$; Fig. 1a, Table 1), several other aspects of the wing morphology also differ between species, including wing length ($F_{3,920} = 361.54, P < 0.0001$), wing width ($F_{3,920} =$

1366.9, $P < 0.0001$) and shape (Fig. 1a, Table 1). In all the species, all traits were positively correlated with each other although the magnitude of the correlations differed between traits and species (Fig. 1b). The wing morphospace (Fig. 1c) shows that divergence among these species has not only involved wing patch length, but also wing width and wing length. The four different groups had “gaps” in between them with respect to wing patch length consisting of 4–5 mm, whereas wing length and wing width were less variable (Table 1; Fig. 1). The wing morphological traits also differed between populations within species ($F_{0.5,918} = 1366.9, P < 0.0001$, for wing patch, and $F_{0.5,918} = 6.0693, P < 0.0001$ for wing length, $F_{0.5,918} = 30.654, P < 0.0001$ for wing width).

Table 1 Wing trait means (and standard errors) for the four study species (populations pooled)

| | Wing length | Wing width | Wing patch length |
|--------------------------------------|---------------|---------------|-------------------|
| <i>Calopteryx splendens</i> | 30.05 (0.042) | 9.77 (0.014) | 15.89 (0.066) |
| <i>Calopteryx xanthostoma</i> | 29.32 (0.079) | 9.55 (0.047) | 19.46 (0.088) |
| <i>Calopteryx virgo meridionalis</i> | 29.73 (0.098) | 10.77 (0.044) | 24.03 (0.097) |
| <i>Calopteryx virgo virgo</i> | 32.08 (0.059) | 11.59 (0.027) | 29.02 (0.081) |

Mean and standard errors are calculated from pooled study populations for each species

Divergence in Phenotypic Covariance Structure of Wing Morphology

The comparisons between the \mathbf{P}_{\max} of the different populations are shown in Fig. 2. There was only weak or moderate divergence in covariance structure between populations of the same species (mean: 7.62° , bootstrap 95% confidence intervals: [0; 15.63]), and this divergence was significantly lower than divergence among populations of closely related species ($P < 0.001$; mean: 30.54° , bootstrap 95% confidence intervals: [23.48; 37.60]). Divergence between populations of the same species was also significantly less than divergence between populations of distantly related species ($P = 0.03$; mean: 17.81° , bootstrap 95% confidence intervals: [13.08; 22.55]) (Fig. 2a). Finally, divergence among populations of closely related species was significantly greater than divergence among populations of distantly related species ($P = 0.01$).

Among populations of distantly related species, geographically close populations showed a quite conserved covariance structure in between them (mean: 8.71° , bootstrap 5% confidence intervals: [5.32; 12.10]) whereas populations which were more geographically distant were significantly more diverged in their covariance structure ($P < 0.001$, mean: 28.94° , bootstrap 5% confidence intervals: [23.84; 34.04]).

The comparisons of vector correlations among the same population categories are shown in Fig. 3. There was a significant influence of taxonomic level involving the different pairwise comparisons (Fig. 3a; $F_{2,33} = 7.49$, $P = 0.002$). There was only weak or moderate divergence in covariance structure between populations of the same species (mean: 0.94, 95% confidence intervals: [0.91, 0.97]), and these populations were more similar than populations of closely related species were ($P = 0.057$; mean: 0.86, 95% confidence intervals: [0.83; 0.90]) or populations of distantly related species ($P = 0.0015$; mean: 0.83, 95% confidence intervals: [0.80; 0.87]) (Fig. 3a). Finally, divergence among populations of closely related species was similar to divergence among populations of distantly related species ($P = 0.449$). Among populations of distantly related species, geographically close populations,

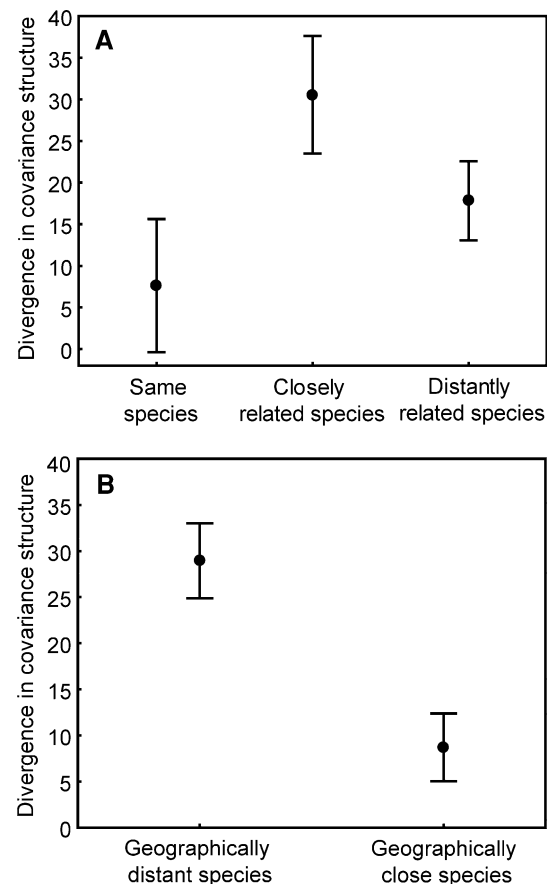


Fig. 2 Divergence in the covariance structure of wing morphology as measured by the orientation of the first eigenvector of their \mathbf{P} -matrices, \mathbf{P}_{\max} . **a** Comparisons of the \mathbf{P}_{\max} orientation at different taxonomic levels between populations of the same species, between populations of closely related species (subspecies or sister species) and between populations of distantly related species. Between populations of the same species, there has only limited divergence in wing covariance structure compared to the divergence between populations of closely related species ($P < 0.001$) and between populations of distantly related species ($P = 0.03$). Divergence between populations of distantly related species is only moderate, and significantly lower than between populations of closely related species ($P = 0.01$). **b** Comparisons of the \mathbf{P}_{\max} orientation of populations of distantly related species, categorized into geographically close or distant populations. Populations of distantly related species which co-occur in the same region have a more similar covariance structure than populations between distantly related species that are more widely geographically separated ($P < 0.001$)

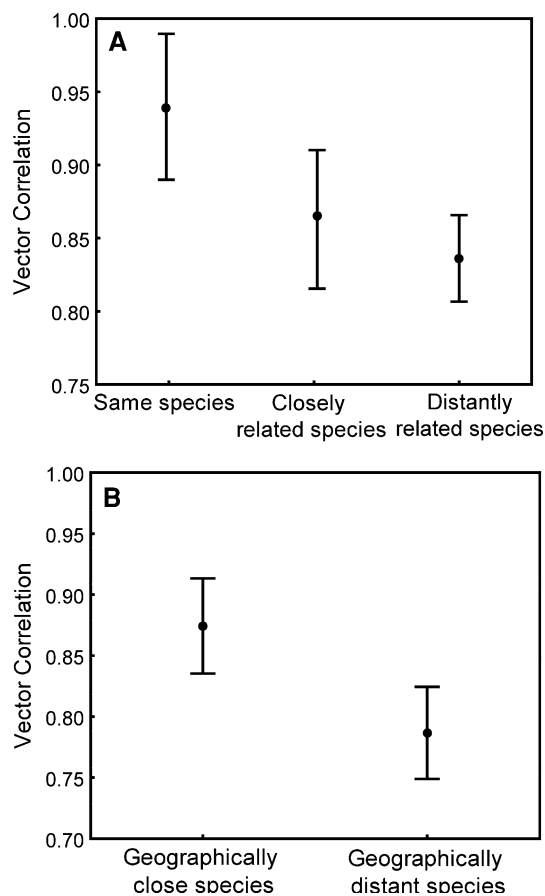


Fig. 3 Divergence in the covariance structure of wing morphology as measured by the correlation of the vector response to 10,000 random skewers (Revell 2007). **a** Comparisons of the correlation of the vector responses of **P** subject to 10,000 random skewers at different taxonomical levels between populations of the same species, between populations of closely related species (subspecies or sister species) and between populations of distantly related species. Between populations of the same species, there was only limited divergence in wing covariance structure compared to the divergence between populations of closely related species ($P < 0.057$) and between populations of distantly related species ($P = 0.0015$). However, divergence in covariance structure between populations of distantly related species did not differ from divergence between populations of closely related species ($P = 0.449$). **b** Comparisons of the correlations of the vector responses of **P** subject to 10,000 random skewers of populations of distantly related species, categorized into geographically close or distant populations. Populations of distantly related species which co-occur in the same region have a more similar covariance structure than populations between distantly related species that are more widely geographically separated ($P < 0.005$)

covariance structure responded in similar ways to the random skewers (mean: 0.87, 5% confidence intervals: [0.85; 0.89]) whereas populations which were more geographically distant were significantly more divergent in their covariance structure ($F_{1,18} = 10.12$, $P = 0.005$, mean: 0.79, 5% confidence intervals: [0.72; 0.85]). Finally, we found that the amount of divergence in covariance structure was significantly correlated with the amount of divergence

in wing patch length (Fig. 4; $r = 0.49$; $P = 0.010$), but not with the amount of divergence in wing length (Fig. 4; $r = -0.132$; $P = 0.443$) or wing width (Fig. 4; $r = 0.223$; $P = 0.181$).

Discussion

Recent field studies on calopterygid damselflies have shown that the male wing traits, including the melanized wing patch are targets of both sexual (Siva-Jothy 1999; Svensson et al. 2004; Tynkkynen et al. 2004; Svensson et al. 2006) and natural selection (Svensson and Friberg 2007). However, these selective pressures differ in strength and sometimes even in direction between different species (Tynkkynen et al. 2004; Svensson and Friberg 2007). Qualitative and quantitative differences in selection pressures on wing morphology are thus likely to be context-dependent upon local environmental conditions such as avian predation risk and the relative frequency of sympatric species (Svensson and Friberg 2007).

Here, we have studied interspecific differences in these wing traits in four different species of this genus. At the multivariate level, we note that these different species do certainly not occupy the entire wing morphospace, but form a weak continuous “ridge” with different degree of overall melanization (Fig. 1c), although we also found significant variation between populations within the same species (Table 1). The overall phenotypic divergence in wing morphology might partly reflect the effects of allometry and size, as indicated by the positive covariances of the different wing traits (Fig. 1). Two of the three traits we measured in this study are associated with overall damselfly size (wing length and wing width) and for these two traits the positive correlations are not necessarily adaptive but might simply reflect overall size. The third trait, wing patch size, is, however, different and is not correlated to overall size. Two of the taxa in this study (*C. v. virgo* and *C. v. meridionalis*) are only slightly larger than the two other taxa (*C. splendens* and *C. xanthostoma*), yet the amount of wing melanization differs dramatically (Fig. 1). Clearly, these striking interspecific differences in a male secondary sexual character cannot be simply attributed to overall effects of size and allometry.

We also note that the morphospace is entirely empty in the region with wide and short wings and with entirely melanized wing area (Fig. 1c). This empty region in morphospace could potentially reflect developmental or functional (e.g., flight) constraints (Willmore et al. 2007; Nijhout 2008; Marden 2008). However, it is more likely that this empty region instead is a result of ecological selection pressures, such as an increased predation risk that is associated with short, wide wings and an entirely

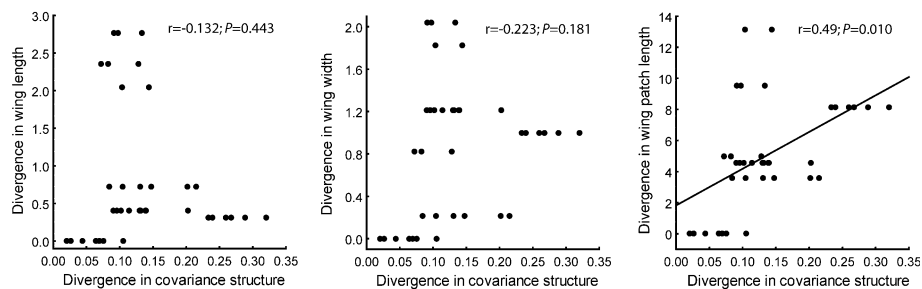


Fig. 4 The relationship between the amount of divergence in overall wing covariance structure and the different wing traits. There was a significant correlation between divergence in covariance structure and

divergence in wing patch length (Mantel test, 10,000 permutations) but not with wing length or wing width

melanized wing area (Svensson and Friberg 2007). Whether empty regions in morphospace in general reflect developmental/evolutionary constraints or whether they reflect ecology is still a controversial issue (McGhee 1999).

Our previous work on predator-mediated natural selection in *C. splendens* and *C. virgo* showed that avian predation favored shorter wings in both these species, but wider wings in *C. splendens* and narrower wings in *C. virgo* (Svensson and Friberg 2007). This might indicate the existence of an “optimal” (intermediate) wing width. We have also found evidence of correlational selection involving wing width and wing length that was remarkably similar in both these species (Svensson and Friberg 2007). Avian predation also seemed to favor a reduction of wing melanization in both these species, particularly in the more exaggerated species *C. virgo* (Svensson and Friberg 2007). Thus, our previous work is certainly consistent with the notion that the empty region of morphospace (Fig. 1), can at least partly be attributed to ecological factors. However, it should be emphasized that predation risk varies dramatically in space, both at the local scale of populations and at a broader geographic scale across Europe (S. Kuchta et al., unpubl. data).

This wing morphospace is also interesting in the context of the phylogeny of calopterygid damselflies (Dumont et al. 2005). The two *C. virgo* subspecies (which belong to a group that radiated 5.7 Mya ago (Dumont et al. 2005)) occupy the upper left part of the morphospace and have large wing widths, lengths, and wing patch lengths. In contrast, the lower right part of the morphospace (smaller wing width, length, and wing patch length) is occupied by the two sister species *C. splendens* and *C. xanthostoma*. These two latter calopterygids belong to another group that radiated 3.7 Mya ago (Dumont et al. 2005). We also note that, although there are highly significant differences between the four species for all three wing traits (Fig. 1a, b), there is also variation between different populations within each species (Figs. 1c and 2a; Table 1). Such intra-specific between-population variation might be associated with divergent sexual selection pressures that differ in sign

and magnitude between closely located populations (Svensson et al. 2004, 2006). This intraspecific variation is, however, as much reflected in wing length and wing width, as with wing patch length (Fig. 1c, Table 1).

In contrast, the main species-diagnostic character in *Calopteryx* is the amount of wing melanization (Fig. 1a), rather than the metric wing traits. This is also the trait that seems to almost entirely influence sexual isolation between the different species in this genus (Svensson et al. 2007). Because of its importance in sexual isolation between species, patch length might be subject to stronger divergent selective pressures than other traits when species are sympatric (Svensson et al. 2007). Divergence in wing patch length is also significantly correlated with the amount of divergence in overall covariance structure (Fig. 4). Strong divergent selection on wing patch length might have decreased the overall stability of the phenotypic covariance structure and made it possible for wing patch length to diverge in different directions. The fact that wing patch length was correlated with overall covariance structure divergence, but not wing length and wing width, is also interesting in terms of the implications for sexual isolation and speciation. Whether sexual isolation in the genus *Calopteryx* is selected per se or whether it evolves only as a correlated response to selection on other traits, the strong link between overall covariance structure and a sexual isolation character like wing patch length could potentially have some important evolutionary consequences (Fig. 4).

Overall, there is a positive correlation between the three traits across all the four calopterygid species (Fig. 1b, c). It is unlikely that all these correlations between the melanized wing-patch and the other wing traits simply reflect developmental associations due to overall size, since melanin production in insects is most likely governed by an entirely different set of genes than the genes governing wing length (Zera et al. 1998; True et al. 1999; Zera 2006).

However, there could still be some limited role for allometry here, even though wing patch size might be only weakly developmentally coupled to wing length and wing width. This is because, the total melanin area of the wing

(and hence the signal strength), can be reduced (or increased) in three different ways: by reducing the size of the patch itself, by reducing wing width or by reducing wing length. The optimal solution to this is likely to depend on both the signs and magnitudes of directional and correlational selection on these three wing traits. It is quite possible, perhaps even likely, that these phenotypic correlations might partly have been influenced and shaped by correlational selection (Sinervo and Svensson 2002). These correlations might thus not only reflect the effects of shared developmental pathways constraining the overall wing phenotype.

Once reproductive isolation has been achieved, there might be potential for greater divergence in covariance structure if gene flow constrained divergence prior to the completion of sexual isolation (Schluter 1996; Eroukhmanoff and Svensson 2008). Therefore, it is perhaps not too surprising that comparisons between populations of the same species reveal a low degree of divergence compared to comparisons between closely and distantly related species (Fig. 2a). The extent of divergence between populations of the same species is lower in the covariance structure of the wing morphology (average difference in \mathbf{P}_{\max} orientation: 7.62°) than it is in overall morphology, as found in our previous study (33.89°) (Eroukhmanoff and Svensson 2008). This indicates that the covariance structure of the wing morphology might be evolutionarily conserved to a greater extent than the covariance structure of other morphological traits. Presumably, this effect might arise from stronger genetic canalization and/or low variability in the developmental pathways affecting wing morphology (Willmore et al. 2007; Polly 2008; Nijhout 2008) compared to the other traits or stronger multivariate stabilizing selection on wing morphology that ultimately affects flight ability (Marden 2008).

While it is perhaps not too surprising that we found greater divergence in covariance structure between than within species (Frankino et al. 2005; Eroukhmanoff and Svensson 2008), the divergence of the covariance structure did not apparently increase in a monotonic way with the taxonomical level at which the comparisons were made. This is because there is no evidence for further exaggerated divergence with increasing phylogenetic distance (Figs. 2a and 3a). The lower divergence in the orientation of \mathbf{P}_{\max} between populations of distantly related species, compared to that between closely related species was unexpected since the potentially constraining effects of gene flow should be weak or even non-existent between these reproductively isolated taxa. If anything, we would expect greater similarity in the covariance structures between closely related species such as *C. splendens* and *C. xanthostoma*, which are known to hybridize in southern France and were until quite recently only considered to be subspecies (Corbet 1999). If divergence of covariance

structures at the interspecific level was constrained by hybridization, as suggested by Schluter (1996), we would expect increasing divergence with increasing phylogenetic distance, rather than the “bell-shaped” pattern we found here (Fig. 2a). However, we note that this pattern was supported when the overall covariance structure was investigated, and we found no effect of taxonomical level in how the covariance structure biased the response to selection among different species (Fig. 3a).

Nonetheless, these results indicate that there is high rate of divergence in the covariance structure of the wing morphology in the early stages of radiation and between recently separated taxa, at least similar to the rate of divergence observed for distantly related species. This might reflect the importance of wing morphology as a reproductive isolation mechanism between recently diverged and incipient species. Lack of clear phylogenetic signals on phenotypic divergence in covariance matrices has actually been demonstrated in other studies. For example Goodin and Johnson (1992) and Steppan (1997) found stronger divergence in covariance structure between subspecies than between distant species. Steppan (1997) suggested that this effect could arise through three different factors: gene flow, stabilizing selection, or environmental variation.

By further comparing the covariance structure of distantly related species, we also found some indications (Figs. 2b and 3b) that convergence in the orientation of \mathbf{P}_{\max} or in the overall covariance structure has occurred between populations of species that coexist in the same geographical area. Indeed, geographically close populations had significantly less divergence than geographically distant species (Fig. 2b) and the average \mathbf{P}_{\max} deviation (8.71°) was similar to that at the intraspecific level (Fig. 2a; 7.62°).

Based on similar findings, Riska (1985) suggested that local long-term stabilizing selection could result in convergence, diminishing interpopulational divergence at the local level. It was also suggested that the maintenance of a “developmental network” might constrain covariance structures over the long term, while enabling different modifications at the trait levels in response to local selection (Riska 1985). The maintenance of such a developmental network across the different species studied here might possibly be illustrated by in the continuum in the wing morphospace (Fig. 1b). Wing length and wing width covaried positively (Fig. 1b). These phenotypic correlations might also reflect ancestral genetic correlations shared across species which are conserved in the entire genus. This is plausible since the species in this study are considered to be evolutionarily relatively young (probably established during the last Pliocene–Pleistocene glaciations (Dumont et al. 2005)).

Our study has involved populations located in Spain and Sweden where environmental differences are quite large. This wide sampling area might explain the strong effects of geography on the covariance structures of wing morphology. In dipterans, wing morphology rapidly evolves as a response to latitudinally varying selection pressures, and temperature seems to play a key role in mediating these selection pressures (Huey et al. 2000; Debat et al. 2003). Furthermore, it has been recently suggested that selection linked to thermoregulation properties could also influence the degree of melanization in insects (Clusella-Trullas et al. 2007). These results might thus reflect weak environmental canalization in wing morphology (Wagner et al. 1997; Debat and David 2001), which predicts similar phenotypes under identical environmental conditions, even if the underlying genotypes differ. Wing morphology in other insects is extremely sensitive to environmental conditions, and the similarities between geographically closely located species could partly reflect phenotypic plasticity to similar temperature environments (Debat et al. 2003). Recently, it has actually been proposed that the conservative role of developmental pathways in wing morphology in insects might be less important than previously thought and that natural selection could be responsible for much of the patterns of divergence in wing morphology (Frankino et al. 2005; Debat et al. 2006). The results in this study are certainly consistent with such a scenario where selection plays a major role.

In conclusion, *Calopteryx* damselflies are excellent model organisms to study phenotypic integration and covariance structure because of their wide geographic distributions and because they have diverged extensively in different wing morphological traits. The several recent field studies on different species of this genus have also clearly shown that wing morphology in *Calopteryx* are targets of multiple selection pressures (Tynkynen et al. 2004; Svensson and Friberg 2007; Svensson et al. 2004, 2007). Here, we have showed that divergence in the phenotypic covariance structure of these traits has mainly taken place at the interspecific level, yet species that are phylogenetically distant seem to have converged to similar phenotypic states when occupying similar environments. We have also shown that wing patch length, a trait subject to several different types of selective pressures, has diverged in a correlated fashion with the overall covariance structure, in contrast to other wing traits. Strong diversifying selection on single traits might therefore decrease the overall stability of the covariance structure, as it has recently been suggested (Arnold et al. 2008). This last finding underscores the important role of the local environment in affecting patterns of phenotypic covariance, irrespective if it results from ecologically similar selective environments (Schluter 2000), developmental plasticity (West-Eberhard 2003) or interactions between these both factors.

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