A role for ecology in male mate discrimination of immigrant females in Calopteryx damselflies?

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Sexual selection against immigrants is a mechanism that can regulate premating isolation between populations but, so far, few field studies have examined whether males can discriminate between immigrant and resident females. Males of the damselfly *Calopteryx splendens* show mate preferences and are able to force pre-copulatory tandems. We related male mate responses to the ecological characteristics of female origin, geographic distances between populations, and morphological traits of females to identify factors influencing male mate discrimination. Significant heterogeneity between populations in male mate responses towards females was found. In some populations, males discriminated strongly against immigrant females, whereas the pattern was reversed or nonsignificant in other populations. Immigrant females were particularly attractive to males when they came from populations with similar predation pressures and densities of conspecifics. By contrast, immigrant females from populations with strongly dissimilar predation pressures and conspecific densities were not attractive to males. Differences in the abiotic environment appeared to affect mating success to a lesser degree. This suggests that male mate discrimination is context-dependent and influenced by ecological differences between populations, a key prediction of ecological speciation theory. The results obtained in the present study suggest that gene-flow is facilitated between ecologically similar populations. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 506–518.

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INTRODUCTION

Populations might be unable to adapt locally if they experience strong gene flow from populations that inhabit different ecological environments (Kirkpatrick & Barton, 1997; Schluter, 1998; Schluter, 2001; Gavrilets, 2004). However, if immigrants experience lower survival upon reaching the foreign habitat, then the likelihood of inter-population matings will be reduced (Nosil, Crespi & Sandoval, 2003). This mechanism has been termed 'immigrant inviability', and leads to reduced encounters and mating opportunities between individuals from divergently adapted populations. This mechanism also reduces the likelihood that successfully mated immigrant individuals will

survive long enough to produce offspring or to mate repeatedly. Several studies have used transplant experiments to investigate the strength of this mechanism in nature (Hendry, 2001; Crispo *et al*., 2006), and some have indeed reported significant natural selection against immigrants (Schluter, 2000).

Another process that can lead to a reduction in fitness of individuals from other populations is sexual selection against immigrants (Via, Bouck & Skillmann, 2000; Nosil & Crespi, 2004). Theoretical models show that sexual selection against immigrants can be a powerful mechanism to reduce or prevent gene flow between populations with different ecologies, and reproductive isolation can be achieved by strong divergent selection in fewer than 100 generations (Kondrashov & Kondrashov, 1999). Strong mate preferences for or against immigrant or resident mates can hence act to regulate the 'inbreeding/

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outbreeding' balance between populations that are connected by occasional gene flow.

The vast majority of studies on sexual selection, and thus far all studies of sexual selection on immigrants, have focused on female mate choice of males. This partly reflects the traditional view that female gamete investment is higher than male gamete investment, as a result of differences in the costs associated with the making of eggs versus sperm (Bateman, 1948; Trivers, 1972). However, it has been recognized that male choice can also be costly and that, in several insect species, males actually discriminate as strongly as females (Bonduriansky, 2001: *Drosophila melanogaster* and *Agrotis segetum*; Svensson *et al.* 2007 and; Wellenreuther, Tynkkynen & Svensson, 2010: *Calopteryx splendens* and *Calopteryx virgo*).

Strong male mate preferences are further facilitated when males suffer high fitness costs of reproduction as a result of physiological, temporal, and ecological costs, which are particularly common in short-lived animals such as insects (Bonduriansky, 2001). For example, a major cost of male mate choice in insects is usually related to courtship, which can be energetically expensive (Judge & Brooks, 2001), and risky in terms of attracting predators (Kotiaho, 2001). Similarly, costs relating to sperm production or energetic costs as a result of male–male competition and territory defence (Härdling, Gosden & Aguilee, 2008; Lemaître *et al.*, 2009) can limit male reproduction, and might result in the strategic allocation of male mating effort towards females and cryptic male choice (Wedell, Gage & Parker, 2002). Such energetic costs are a realistic trade-off in insects because females of many species are mated repeatedly during the reproductive season, thus drastically increasing the total investment of males.

The banded demoiselle (*C. splendens*) is a common damselfly in most parts of Europe and co-occurs over most of its geographic range sympatrically with the closely-related congener the beautiful demoiselle (*C. virgo*) (Corbet, 1999; Askew, 2004). Populations of *C. splendens* and *C. virgo* are found in a geographic mosaic of allopatric and sympatric populations in southern Sweden (Svensson *et al.*, 2004; Tynkkynen, Rantala & Suhonen, 2004; Svensson, Eroukhmanoff & Friberg, 2006). Individuals of *C. splendens* disperse in a response to habitat quality and population density (Stettmer, 1996); hence, populations are connected by the occasional mixing of different populations (Svensson *et al.*, 2004). This population structure is useful for studying premating isolation because the different environments can create contrasting selection pressures, whereas the close proximity of populations increases the opportunity for gene flow. A recent study by Svensson *et al.*

(2006) on *C. splendens* quantified premating isolation between populations by measuring female mate responses towards resident and immigrant males. The study showed that immigrant males had lower courtship success than resident males, and this was interpreted as evidence for incipient sexual isolation between populations (Svensson *et al.*, 2006). However, the relative importance of female choice in promoting gene flow between populations of *C. splendens* remains unclear because the effects of female choice in reducing gene flow could be counteracted by forced copulations by males. Several studies have shown that *C. splendens* males can force females into precopulatory tandems, and these tandems rarely break down without copulation (Cordero, 1999; Cordero & Andrés, 2002). Therefore, male choice of females might be an equally or even more important factor in preventing gene flow between populations. Moreover, recent studies on *Calopteryx* males (Svensson *et al.*, 2007; Wellenreuther *et al.*, 2010) have demonstrated that males can distinguish between different female phenotypes and, in some instances, will even avoid a mating opportunity. In addition, males of *C. splendens* suffer intense male–male competition for oviposition territories, and repeated copulations during the mating season are likely to affect sperm availability. This is because males cannot produce limitless amounts of sperm (Nakatsuru & Kramer 1982; Wedell *et al.*, 2002), in particular when high population density leads to repeated matings and sperm depletion (Arnqvist & Danielsson 1999). In such cases, sperm production costs in insects can be high enough to cause a reduction in life span (Van Voorhies 1992). For these reasons, male mate choice in *C. splendens* might be a key factor that can potentially regulate gene flow between populations.

In the present study, we examine the role of male mate choice in premating sexual isolation between populations of *C. splendens* in southern Sweden. Both local and immigrant females were presented to local males in 18 populations (nine reciprocal pairs) and, for each female, the male courtship response was recorded and the degree of premating isolation calculated. Male responses towards females were then related to the ecological origins of each pair, female morphological traits, and geographic distance. This allowed us to test whether the ecological distance between populations affects the degree of sexual reproductive isolation, as has been predicted by empirical (Hendry, 2001; Rundle & Nosil, 2005) and theoretical studies (Dieckmann & Doebeli, 2000; Doebeli & Dieckmann, 2003). Ecological speciation theory predicts that the degree of reproductive isolation should be a function of ecological differences between populations (Schluter, 1998; Rundle & Nosil,

2005), which separates this theory from its many non-ecological alternatives (Coyne & Orr, 2004).

MATERIAL AND METHODS

STUDY SPECIES

The distribution of *C. splendens* occurs as far south as the Mediterranean in France, and has a northern range limit that coincides with the classical biogeographical boundary 'Limes Norrlandicus' in central Sweden (Aguilar & Dommanget, 1998; Askew, 2004). Mark–recapture studies have demonstrated no significant difference in the dispersal of *C. splendens* males and females, although they have identified habitat quality and population densities as causative agents in promoting dispersal between populations (Stettmer, 1996). Furthermore, dispersal distances between *C. splendens* populations were found to be large, with the maximum measured dispersal distance being 4 km over a 24-h period (Stettmer, 1996). Throughout most of their range, *C. splendens* co-occurs sympatrically with the closely-related species *C. virgo*, and, as a result of species-specific habitat differences, the two *Calopteryx* species can be found in a geographic mosaic of sympatric and allopatric populations (Wellenreuther *et al.*, 2010).

In southern Sweden, the emergence of adults takes place from late May and proceeds mainly until June (Svensson *et al.*, 2004; Svensson *et al.*, 2006). Immediately after emergence, but prior to sexual maturity, teneral individuals spend most of their time away from the river to forage and mature (Corbet, 1999). After sexual maturation, both males and females return to the river to mate. Both mating and egg oviposition take place exclusively at or near the water surface (Corbet, 1999). Males of *C. splendens* defend riverine territories consisting of a few perching sites where females visit to mate and oviposit eggs on submerged plants. Males are polygynous and territorial and combat with other males for access to females and suitable sites for oviposition (Plaistow & Siva-Jothy, 1996). In *C. splendens*, females can be forced into precopulatory tandems, and these tandems rarely break down without copulation (Cordero, 1999; Cordero & Andrés, 2002). Previously, amplified fragment length polymorphisms (AFLPs) have been used to estimate molecular differentiation between *C. splendens* populations in southern Sweden. The study showed that the average differentiation between populations was with a F_{ST} value of 0.04 weak to moderate (Svensson *et al.*, 2004). Another AFLP study on *C. splendens* populations from Eurasia found an overall F_{ST} value of 0.28 (Sadeghi *et al.*, 2010), which is not surprising given the much larger geographic distances between populations.

Together, these two studies suggest moderate to high gene flow between populations (Svensson *et al.*, 2004; Svensson *et al.*, 2006), and we are currently investigating this in more detail using novel microsatellite markers for this species.

DATA COLLECTION

To quantify male courtship responses and to estimate the degree of premating sexual isolation between populations, resident and immigrant females were presented to local males at seven study populations during the reproductive season in 2007 and 2008. The study populations were located in southern Sweden with distances between populations spanning from 9–106 km (Fig. 1). The seven study populations included some populations that were used in a previous study on female mate choice in *C. splendens* conducted by Svensson *et al.* (2006), namely 'Klingavälsåns Naturreservat' (River: Klingavälsån; 55.6384, 13.54142), 'Värpinge' (River: Höje Å; 55.7022, 13.14379), 'Omma' (River: Åsumsån; 55.6506, 13.66987), Världs Ände (River: Klingavälsån; 55.62178, 13.62521), and 'Härnas' (River: Mörrumsån; 56.33039, 14.70557). In addition, we used populations at 'Näsby' (River: Tolångaån; 55.65782, 13.82492) and 'Sövdemölla' (River: Klingavälsån; 55.60447, 13.65638) as two new populations in our study. A total of nine reciprocal population comparisons were conducted, resulting in a total of 18 populations for which resident and immigrant female courtship success could be quantified.

For the courtship experiments females were caught with hand nets and kept in a holding container until the start of each presentation session. Only adult females with paper-like, crisp wings and firm veins that were in close proximity to the river were used for the courtship presentations (Plaistow & Tsubaki, 2000), whereas all immature females were immediately released after capture. The presentations were carried out by tying a 0.5-m long thread around the thorax of the female, and then attaching the thread to a 1.5-m long bamboo stick. Each tethered female was allowed to fly in close proximity to one of the target males, which were, in all cases, territorial males that were adjacent to or flying over the river. All males that were selected for the presentation experiments were not involved in combat and were resting on vegetation when the presentations started. Courtship experiments were exclusively performed between 09.00 h and 17.00 h, on calm and sunny days without rain. The courtship responses of males were quantified on a four-degree nominal scale, where 0 represents escape, 1 represents approach, 2 represents courtship, and 3 represents clasping. A total of 288 females were presented, and each female was pre-
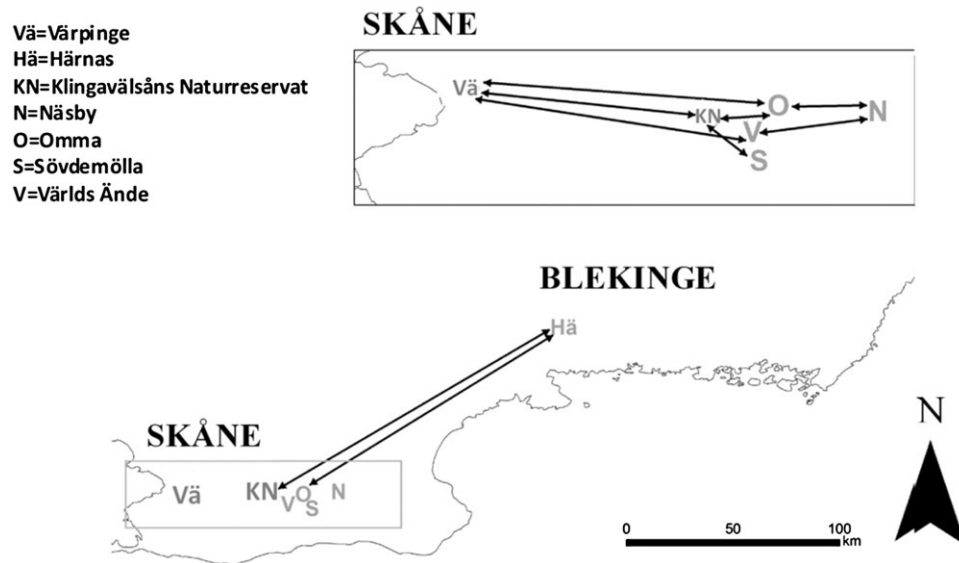
Figure 1. Map of study locations in southern Sweden (regions of Skåne and Blekinge), showing the relative position of the seven populations: KN, Klingavälsåns Naturreservat (Klingavälsån); O, Omma (Åsumsån); Vä, Värpinge (Hoje Å); V, Världs Ände (Klingavälsån); S, Sövdemölla (Klingavälsån); N, Näsby (Tolångaån); Hä, Härnas (Mörrumsån). Arrows between populations indicate reciprocal transplants.

sented to at least five males at both the origin and non-origin population (mean number of males to which each female was presented: resident population 7.45; immigrant population 7.44). The handling and transportation of the experimental immigrant females was kept at a minimum; however, in some instances, the reciprocal courtship experiment could not be completed in 1 day and, in these cases, females were kept overnight. Females never showed any obvious negative effects of storage and were actively flying during the next day when tethered. This is consistent with the previous study by Svensson *et al.* (2006), which also found no negative effects of handling and transportation on *C. splendens*. The morphology of all presented females was measured subsequently in the laboratory with digital callipers (to the nearest 0.01 mm). Eight morphological characters were measured: thorax length and thorax width, forewing length, forewing width, hindwing length, hindwing width, abdomen length, and total length.

To quantify the ecological characteristics of each population, frequency counts and habitat assessments were conducted (mean per population = 12). All frequency counts were performed by M. Wellenreuther with a wheel pedometer along a 100-m stretch next to the river, and all males and females of *C. splendens* and *C. virgo* were recorded. The habitat was assessed by estimating the degree of forest cover, oviposition sites, the number of open spots on the side of the river that could be used as perches for wagtails,

(*Motacilla* spp.), the main avian predator of *C. splendens* and *C. virgo* (Svensson & Friberg, 2007), the number of avian wagtail predators, and current speed. The amount of forest cover, oviposition sites, and the number of perches for wagtails was categorized from 1–5, with 1 denoting little presence and 5 denoting a maximum abundance of that resource. Current speed was grouped into three categories, with 1 being slow, 2 being medium, and 3 being a fast flowing river. The number of resident pied wagtails was also counted during the damselfly frequency count. The geographic distance between population pairs was estimated with the distance measure tool in ARCMAP, version 9.2 (ESRI).

STATISTICAL ANALYSIS

First, to investigate the effects of habitat and female morphology on the courtship responses, the habitat and morphological distances between populations were calculated. The ecological variables used in the calculation were: the number of *C. splendens* males and females, density of males and females of the heterospecific damselfly *C. virgo*, forest cover, the number of oviposition sites, current speed, the number of wagtail perches, and the number of wagtails sighted per site. Habitat variables were summarized using a principal components analysis (PCA; FACTOR procedure, SAS software, version 9.1; SAS Institute Inc.), and all prior communality estimates were set to 1. The principal axis method was then

used to extract the components. The first three components displayed Eigenvalues greater than 1 and a scree test also suggested that the first three components should be retained. The retained components were rotated using an orthogonal Varimax procedure. An ecological variable was judged to affect a given component if the factor loading was ≥ 0.50 or < 0.50 for the other components. The Euclidean ecological distances were calculated for each reciprocal population comparison (with distances equal to 0 when the female was presented to males at the females' population of origin). Female morphology was summarized for each female by conducting a PCA on all eight morphological variables (FACTOR procedure, SAS software, version 9.1).

Second, premating isolation between populations was investigated using the average male courtship response score towards each female to avoid pseudoreplication as a result of multiple presentations of a female to several males (*sensu* Svensson *et al.*, 2006). Examination of the courtship scores showed that, although they were positively skewed, the residuals of the response scores were normally distributed and there was no mean–variance relationship, justifying the use of parametric statistical tests. A full factorial general linear model (GLM) model with population comparison and female status (immigrant/resident) as categorical predictors and male response as the dependent variable was used to investigate the role of these factors and their interaction on premating isolation between populations. Subsequently, a factorial analysis of variance (ANOVA) in a GLM framework was used to examine the courtship responses of *C. splendens* males towards females for each population pair separately, using STATISTICA, version 8.0 (StatSoft, Inc.) (population pairs, $N = 9$). The courtship response of local males towards immigrant and resident female was entered as the dependent variable, and the population in which the female was presented and female status (immigrant/resident) were categorical predictors. An interaction term between population and species was also included. To further evaluate premating sexual isolation between populations, the differences in courtship scores of immigrant and resident females at a population were used to estimate the extent of sexual isolation between the two female source populations. Premating sexual isolation was calculated for each population comparison (18 population comparisons) by dividing the mean immigrant score by the resident score at a population. The 95% confidence intervals, based on the variation of each single immigrant score, were calculated.

Third, differences in population ecology (i.e. habitat, and female morphology) and distance (i.e. geographic distance) were related to premating isolation between populations to investigate causal relationships. Female courtship scores were analyzed within a GLM framework that simultaneously accounted for repeated measures by treating female ID as a random factor (GENMOD procedure, SAS software, version 9.1). The dependent variable was an ordinal variable using a multinomial logistic regression. The population of origin and the population of presentation were always included in the model (accounting for 16 degrees of freedom), and the unit of repetition was female identity (repetition clusters, $N = 333$; individual mating observations, $N = 4284$). The fixed effects in the model included the Euclidian morphological and ecological distances, and the geographical distance between populations. The model thus tested for the effect of morphology, geographical distance, the distance in predation pressures (first component), the distance in habitat characteristics (second component), the distance in social environment (density of con- and heterospecifics, third component), and the interaction between these distance measures (for details see Results). Chi-square tests were used to assess the significance of effects and type III sum of squares were used in all cases. The model that best explained the data was selected using the lowest Akaike's information criterion (AIC) criterion. We further calculated the ΔAIC values (i.e. Δ_i) to evaluate the relative increase in explanatory power for each model because an individual AIC value, by itself, is not interpretable as a result of the unknown constant (interval scale) (Burnham & Anderson, 2002).

RESULTS

The PCA on the habitat variables resulted in three significant axes which accounted for 63% of the total variance (Table 1). After rotation, the ecological variables were combined (based on their loadings, see Table 1), into three respective groups (Fig. 2). The first component (axis) included four factors; density of *C. virgo* males, the number of oviposition sites, the number of perches for avian predators, and the number of wagtail predators. The first component was therefore mainly interpreted as the 'predation' axis (Fig. 2). The second component was characterized by three factors, namely, density of *C. virgo* females, forest cover, and current stream speed, which was interpreted as the 'abiotic environment' axis (Fig. 2). Finally, the third component consisted of two factors, namely the density of *C. splendens* males and females, which was consequently interpreted as the 'social environment' axis (Fig. 2). These three axes thus summarize the significant habitat differences between the seven study populations used in the reciprocal transplants. The same approach was used to estimate differences in female morphology between

Table 1. Rotated factor pattern and final communality estimates (h^2) from the PCA of the population ecological factors

Ecological factors	First component: Predation	Second component: Abiotic environment	Third component: Social environment	h^2
Density of male *Calopteryx splendens*	0.33	−0.26	**0.65**	0.60
Density of female *Calopteryx splendens*	−0.13	**−0.67**	**0.82**	0.69
Density of male *Calopteryx virgo*	**0.67**	0.31	0.11	0.56
Density of female *Calopteryx virgo*	0.06	**0.59**	0.42	0.53
Forest cover	0.14	**0.77**	−0.32	0.72
Number of oviposition sites	**−0.64**	0.16	0.11	0.45
Current speed	0.06	**0.78**	−0.20	0.65
Number of perches for avian predators	**0.88**	0.02	0.08	0.78
Number of avian predators sighted	**0.73**	0.32	0.05	0.65

The first component was denoted as the predation axis, the second component as the abiotic environment (habitat) axis and the third component as the social environment component. Significant factor loadings are shown in bold.

Table 2. Output of the global factorial general linear model testing the effects of 'population comparison' and 'female status', as well as the interaction between these two factors

	d.f.	SS	MS	F	P
Population comparison	17	34.360	2.021	10.15	< 0.001
Female status (immigrant or resident)	1	1.034	1.034	5.20	0.023
Population comparison × Female status	17	76.987	4.529	22.75	< 0.001
Error	539	107.310	0.190		

populations. However, the calculation of the Euclidean distances for female morphology resulted in one meaningful axis in the PCA, and this axis accounted for 5% of the total variance. The lower amount of variance explained by the morphological PCA is a result of all morphological variables being positively correlated, which is common for morphological measurements. Out of all morphological variables measured, thorax width and thorax length measurements were the variables with the least support, presumably because they also had the largest measurement error (as indicated by the largest standard error of all variables).

The extent of premating isolation between populations showed that male mate responses were significantly affected by both population and female status, as well as by the interaction between female status and population (Table 2). Closer inspection of the male courtship responses revealed large heterogeneity between the 18 different population combinations. In some populations, males did not discriminate between resident and immigrant females, whereas males in other cases discriminated strongly against particular females (Fig. 3). The sexual isolation indices and the associated confidence intervals revealed significant differences between populations (Fig. 3). Specifically, significant male discrimination was present in six out of the 18 population comparisons. In four of these cases, immigrant females obtained courtship scores that were lower than resident females (Fig. 3). In the two remaining cases, immigrant females received higher courtship responses from local males compared to resident females (Fig. 3). Sexual premating isolation is therefore not easily predicted by female status alone, but appears to be influenced by ecological factors unique to populations, geographic distances between populations or by morphological differences between females. This finding was supported by the factorial ANOVAs, in which each population pair was analyzed separately, and where female status had a significant effect on the courtship success in four out of the nine population comparisons, indicating nonrandom mate preferences (Fig. 4). We further found a significant population effect in six of the nine population comparisons (Fig. 4), which reflects differences in male mating propensity. In addition, the presence of four significant interactions between population and female status suggests that the way in which one of the two populations was affected by the female status was different from the other population (Fig. 4). Thus, these results suggest that the extent and type of male mate discrimination differs considerably between study populations.
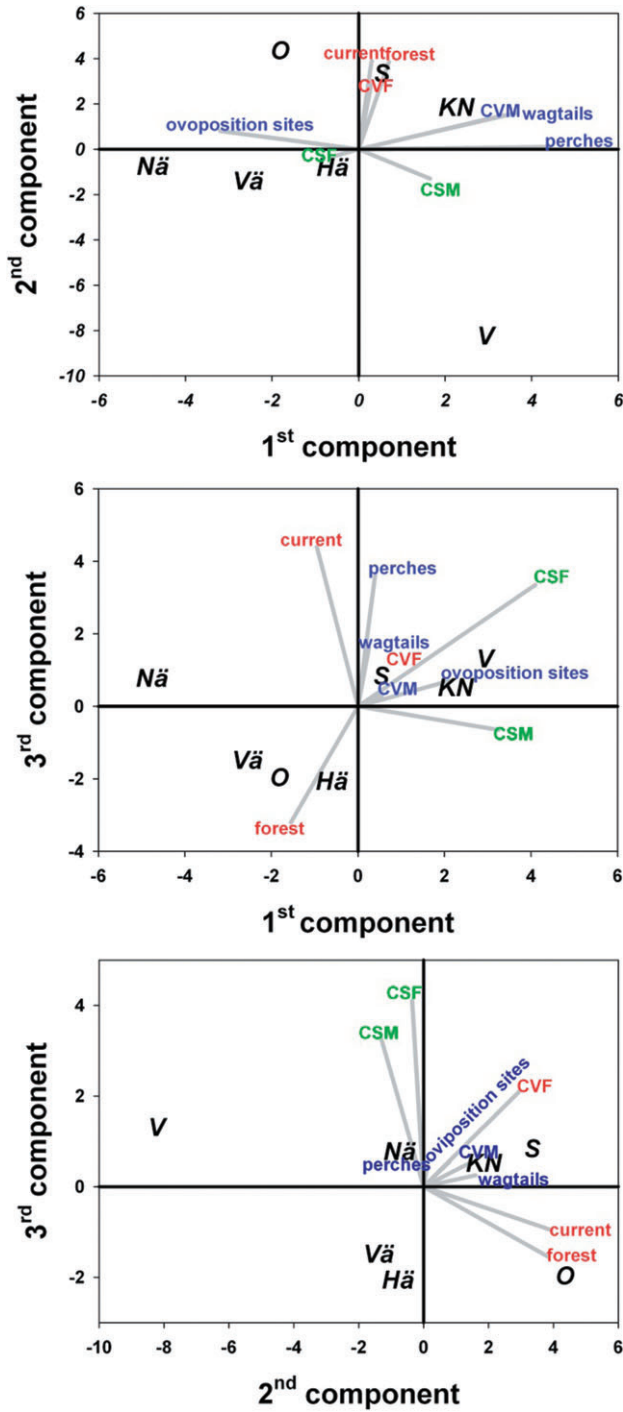
Figure 2. Principal component analysis (PCA) of the ecological differences between sites. Colours denote the three different PCA groups depending on the loadings on the three axes (for the exact loadings, see Table 1). Populations are abbreviated by their first letter (KN, Klingavälsåns Naturreservat; O, Omma; Vä, Värpinge; V, Världs Ände; S, Sövdemölla; Nä, Näsby; H, Härnas).
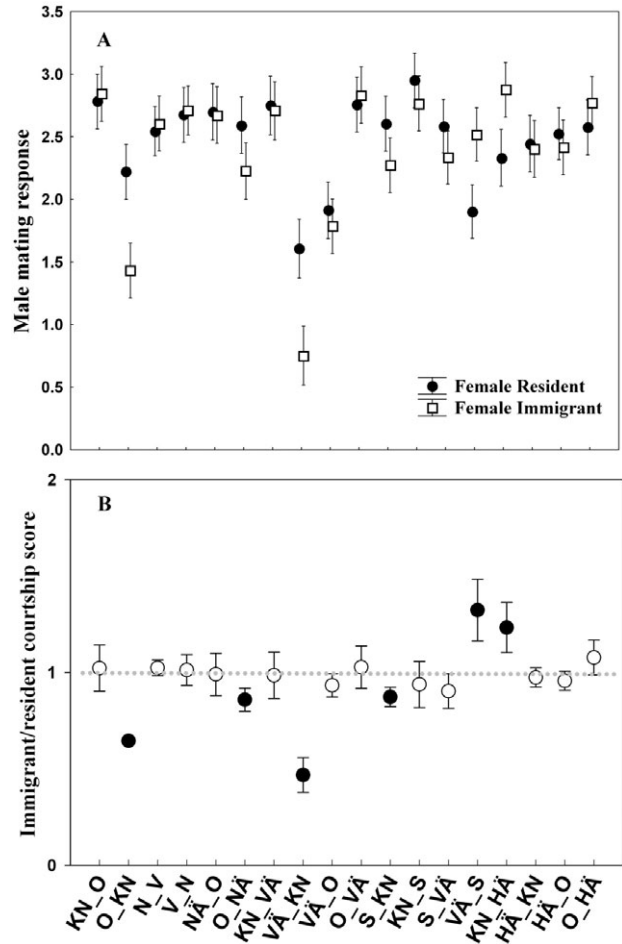

Figure 3. Results of the overall factorial general linear model of male courtship responses towards immigrant and resident females in the 18 different population comparisons and their interaction (A). Differences in courtship success of the same females presented in their population of origin and another population (immigrant/resident score) (B). The dotted line indicates population comparisons where immigrant and resident females have the same courtship success. Error bars indicate 95% confidence intervals. Black circles denote populations with significant premating isolation. Populations are abbreviated by their first letter (KN, Klingavälsåns Naturreservat; O, Omma; VÄ, Värpinge; V, Världs Ände; S, Sövdemölla; NÄ, Näsby; H, Härnas).

In the next step, the ecological differences, female morphology, and geographic distances between populations were related to the extent of premating isolation between populations to identify causal relationships that contribute to premating isolation. The ecological and morphological Euclidean distances, together with the geographic distance between populations, entered the model as fixed effects, and immigrant and resident courtship success entered as a
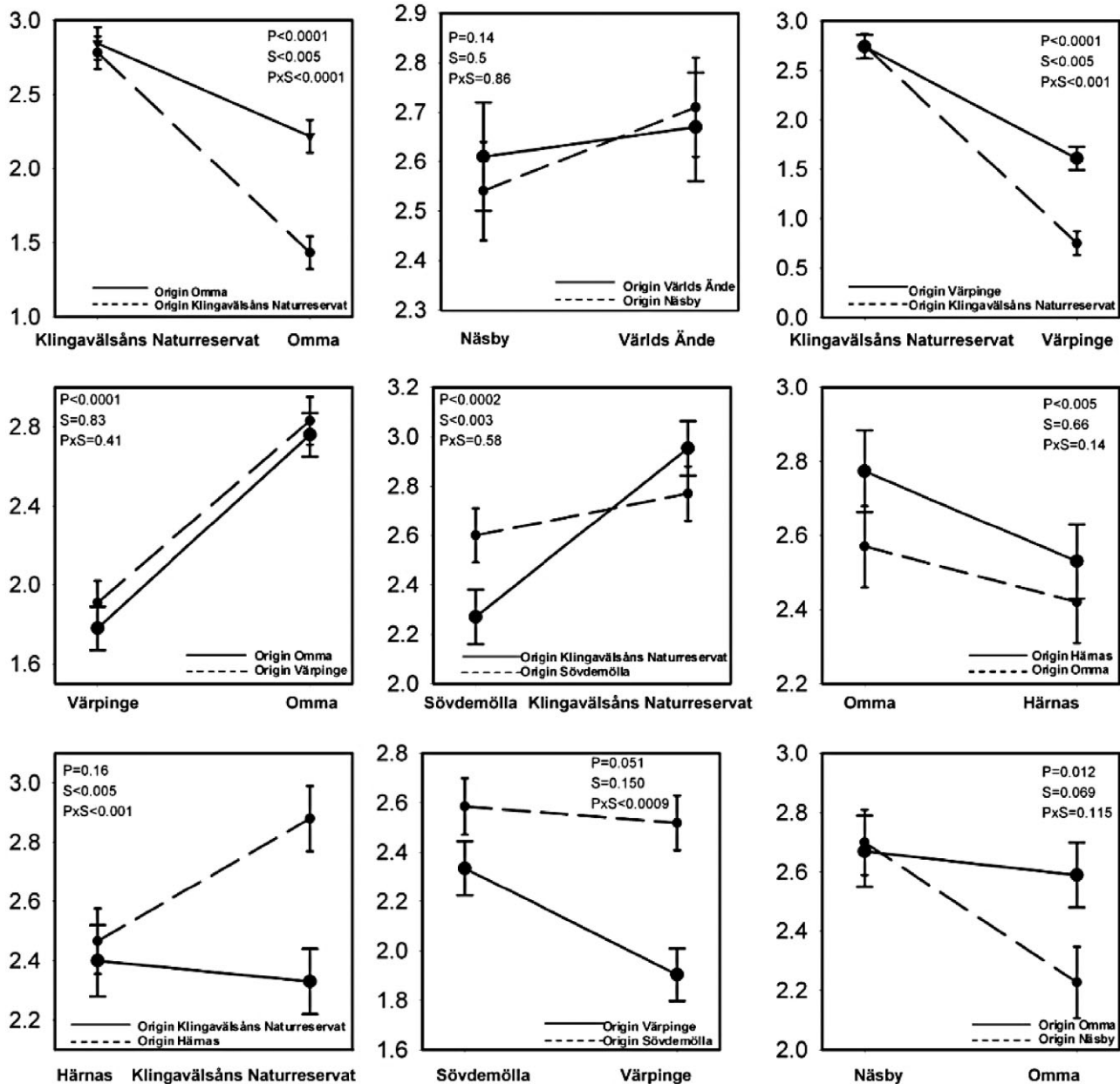
Figure 4. Factorial general linear model per population combination (nine reciprocal transplants) to disentangle the effects of female status, population and their interaction on male courtship responses. P, population effect; S, status of a female (immigrant or resident); PxS, interaction term. Error bars indicate the standard error. Significant results: *$P < 0.05$, **$P < 0.001$. NS, nonsignificant.

dependent factor. The first run of this model showed that the morphology of females had little effect with respect to explaining courtship scores of resident and immigrant females ($P > 0.05$), and the morphological distances were consequently excluded from the global analysis. Population effects were always significant ($P < 0.01$); therefore, these effects were always included in the models. The final generalized model that was chosen had the lowest AIC score (Table 3).

The model that best explained the variation in male mate choice of immigrant and resident females included the effects of the Euclidean distance on the predation (first) and the social environment (third) components, and their interaction, but excluded the abiotic environment component (second), and morphological and geographical distance [GLM: $\chi^2 = 23.04$; d.f. = 1; $N = 333$; $P < 0.0001$; Table 4]. The model showed a negative effect of both the distance on the

Table 3. Results of the generalized linear model testing for the effect of geographical distance (Geodist), the distance in abiotic environment (habitat) characteristics, and the distance in social environment (for the list of variables included in these three ecological distances, see Table 1), and the interaction between these ecological distances

Models without geographical distance (number of parameters)

Model description	AICc	Δ_i
Null model (16)	6147.7	40.8
Predation (17)	6143.6	36.7
Habitat (17)	6149.7	42.8
Social environment (17)	6142.4	35.5
Predation × Habitat (19)	6124.4	17.5
Predation × Social environment (19)	**6106.9**	–
Habitat × Social environment (19)	6138.4	31.5
Predation × Habitat × Social environment (23)	6109.9	3.0

Models with geographical distance (geodist) (number of parameters)

Model description	AICc	Δ_i
Geodist (17)	6148.3	41.4
Predation + Geodist (18)	6131.5	24.6
Habitat + Geodist (18)	6141.6	34.7
Social environment + Geodist (18)	6129.5	22.6
Predation × Habitat + Geodist (20)	6114.2	7.3
Predation × Social environment + Geodist (20)	6109.3	2.4
Habitat × Social environment + Geodist (20)	6122.4	15.3
Predation × Habitat × Social environment + Geodist (24)	6111.1	4.2

$\Delta_i = AICc_i - AICc_{min}$.

The model that best explained the data was selected using the lowest corrected Akaike's information criterion (AICc). AICc scores associated with all tested models are shown. The best model is highlighted in bold.

Table 4. Output from the final generalized linear model (model with the highest AICc score; see Table 2), including the effect of the population of origin and the population where the female was presented to males

Ecological covariates	d.d.f.	χ^2	P
Population of origin	6	76.89	**< 0.0001**
Population of presentation	6	42.63	**< 0.0001**
Distance on the first axis (predation)	1	8.42	**0.0037**
Distance on the third axis (social environment)	1	17.29	**< 0.0001**
Distance on the first axis × Distance on the third axis	1	23.04	**< 0.0001**

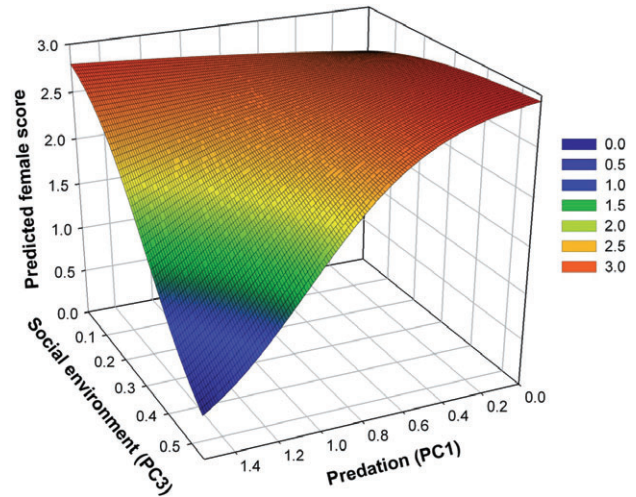d.d.f., denominator degree of freedom.

Figure 5. Surface plot showing the Euclidian distances between populations in the two retained principal components analysis components (x = predation axis; y = social environment axis; for details, see Table 1) in relation to the mating responses that females obtained from males. The colour code (z-axis) quantifies the female score depending on the ecological distance between the population of origin and the population where the female was presented as an immigrant.

predation and the social environment components, and a positive effect on their interaction. A surface plot displaying the combined effects of predation and the social environment on female courtship score is shown in Figure 5.

DISCUSSION

Many species are distributed in discrete populations that are connected by the occasional dispersal of individuals, a condition that will favour local adapta-

tion (Mopper & Strauss, 1998). Dispersal between populations results in encounters between immigrant and resident individuals, which in turn affects mating decisions. It has been suggested that females could benefit from choosing locally adapted males (Proulx, 2001); however, little is known about the role of male mate choice in this context. In the present study, reciprocal transplant experiments were used to investigate whether local males of the damselfly *C. splendens* were able to distinguish between immigrant and resident females. Furthermore, we investigated whether male mate responses can be explained by environmental factors such as the ecological and geographic distances between populations and female morphology. The results obtained in the present study suggest that males can distinguish between immigrant and resident females and that this is reflected by their courtship display. The analyses of male courtship demonstrated that female morphology and geographic distance do not explain variation in male courtship responses, whereas ecological differences between populations had strong and significant impacts on male mate responses (Figs 2, 3).

Svensson *et al*. (2006) used a reciprocal transplant approach (Fig. 3) to investigate female responses to resident and immigrant males in *C. splendens*. Such an experimental approach is derived from similar experimental tests of divergent natural selection (Schluter, 2000), where local adaptation is usually measured as the interaction between population and status (resident/immigrant). In the present study, there was evidence of significant interactions in some of these reciprocal transplants, but not in all of them (Fig. 3). This type of reciprocal transplant approach has been underutilized in studies of premating isolation between populations and it is suggested that it should be used more systematically in future studies. Local females from Klingavälsåns Naturreservat were preferred more strongly by their local males compared to female immigrants from both the Värpinge and Omma populations, which are geographically close (Figs 1, 3). By contrast, female immigrants from the geographically very distant population of Härnäs (Fig. 1) actually elicited stronger mate responses from the local males at Klingavälsåns Naturreservat (Fig. 3). These results emphasize that the geographic origin of females was a weak predictor of female attractiveness, in contrast to the ecological differences between populations (Fig. 5; Tables 3, 4).

The model identified that male courtship choices of immigrant females were high when the females came from a population with similar predation pressures and densities of conspecific damselflies, whereas the abiotic environment of the female population did not influence male mating decisions (Table 2). Conversely, females that originated from populations with dissimilar predation pressures and conspecific densities received the lowest male courtship responses. This result suggests that males might prefer females that are adapted to similar ecological conditions. By choosing females from environments similar to their own, males may improve the overall fitness of their offspring, and the effective gene flow from ecologically divergent populations will be reduced, thereby preventing recombination from breaking up locally favourable gene complexes (Tregenza & Wedell, 2000). Such male choice of (both local and immigrant) females that are adapted to similar ecological conditions might ultimately increase the possibility of speciation (Proulx, 2001) because such a preference would facilitate ecological adaptation and hence increase rates of divergence between populations. Recent theory suggests that sexual selection and spatial variation in environmental conditions might favour evolutionary branching and possibly also speciation (Doebeli & Dieckmann, 2003). It should be noted, however, that our ecological population assessment is by no means complete and that many other ecological variables could be of relevance during mate choice decisions, such as the parasite load or the colour of potential mates. Future studies investigating the role of additional ecological factors affecting male mate choice would be needed to evaluate this further.

By contrast to the significant role of population ecology, differences in external female morphology did not appear to affect male courtship. This suggests that ecological differences between populations are more important in mate choice than morphological differences of females between populations of this species, and that female external morphology is not strongly linked to local ecology. In insects, visual, tactile and olfactory mechanisms can be employed concurrently or sequentially by males to obtain information about females (Bonduriansky, 2001). For example, colour of female wings has been shown to strongly affect species discrimination in *Calopteryx* spp. males (Waage, 1979). Mate choice in some species can also be driven by 'novel' phenotypes, which might increase the success of dispersers relative to residents. Such novel phenotypes can readily arise in ecologically divergent environments as a by-product of adaptations to local conditions (McKinnon *et al*., 2004). For example, work on sexual selection has demonstrated that females of some animals show a preference for males with rare or novel rather than specific phenotypes (guppies: Hughes *et al*., 1999; fruit flies: Singh & Sisodia, 2000), which might in turn impose negative frequency-dependent selection on male phenotypes. Similar negative frequency-dependent processes also

appear to operate with respect to male mate choice of females in several species of damselflies (Svensson *et al.*, 2009). Moreover, work on male guppies has shown that males prefer to court unfamiliar females, and may actively move among schools and among pools in the wild in an attempt to find unfamiliar females (Kelley, Graves & Magurran, 1999). For these reasons, the absence of significant effects of female external morphology on the courtship of *C. splendens* males suggests that males use other cues to assess the attractiveness of females, and to discriminate between immigrant and resident females (Fig. 2).

The fact that males in some populations preferred immigrant over local females (Fig. 2) also suggests that migration of females into neighboring populations does not necessarily result in a mating disadvantage. Studies on great tits (*Parus major*) and Glanville butterflies (*Melitaea cinxia*) show that the immigrant pool is not a random subset of the source population (Hanski & Saccheri, 2006). Such biased migration could impose less of a genetic load, or even increase the amount of advantageous variation in the local phenotype distribution. Thus, although dispersers could have a selective disadvantage in terms of adaptations to the local ecology, they could also have increased mating success in some situations. It should be noted, however, that although sexual selection might favour immigrants over residents in certain situations, gene flow between populations will be reduced if dispersers are considerably maladapted to the local conditions, and thus suffer a higher mortality than locally adapted residents (Rundle & Nosil, 2005). This reduction in gene flow is especially efficient, for example, if dispersers are more prone to die before reproduction (Via *et al.*, 2000; Rundle, 2002; Nosil & Crespi, 2004; Nosil, Vines & Funk, 2005). Under these conditions, realized gene flow will be substantially lower than observed individual levels of dispersal, leading to at least partial reproductive isolation between populations (Crispo *et al.*, 2006). These opposing effects of natural and sexual selection suggest that divergent selective regimes might either increase or decrease gene flow and that they can interact in this process. Experimental manipulations of gene flow would be useful for empirically evaluating the extent and mechanisms by which gene flow constrains adaptation and vice versa (Hendry, Nosil & Riesenberg, 2007), although such experiments provide formidable empirical challenges.

In summary, the results obtained in the present study show that mate preferences of *C. splendens* males are highly context dependent, and the findings also illustrate the dynamic potential of adaptive male mate choice for this species. Courtship responses of male *C. splendens* are strongly affected by the ecologi-

cal distances between populations, and local males prefer females from populations with similar ecologies to their natal populations. The findings thus support a key prediction from ecological speciation theory: that the degree of ecological distance between populations is important and determines the degree of sexual reproductive isolation. Although there has been an increase in studies testing this prediction over the last decade (Schluter, 1998; Schluter, 2000; Rundle & Nosil, 2005), many of these studies have focussed their effort on female mate choice, and not on male mate discrimination. A role for ecology in male mate discrimination of females in these damselflies might appear to conflict with previous arguments, as well as the general view that odonate populations and species might diverge mainly by non-ecological mechanisms, such as sexual selection (McPeek & Gavrilets, 2006). Indeed, a previous study on male *C. splendens* supported this non-ecological view and showed that sexual selection of males was stronger than natural selection, at least during the adult part of the life cycle (Svensson *et al.*, 2006). The results reported from the present study indicate that male choice of females might be more influenced by fecundity-related ecological traits, and not display traits dictated by female choice of males (Bonduriansky, 2001). The present study highlights the need for more studies on male mate choice to investigate the effects of migration on the potential for gene flow, and more explicit considerations of male mate discrimination of resident and immigrant females.

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