Influence of interspecific interference competition on the genetic structure of *Calopteryx splendens* populations

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Understanding the effects of interspecific competition on genetic diversity will deepen our knowledge on species evolution. In the case of *Calopteryx splendens* and *C. virgo*, sympatric damselfly species, interspecific interference competition by *C. virgo* has remarkable effects on territoriality of *C. splendens* resulting in reproductive character displacement. Since territoriality is correlated with phenotype and mating success, we investigated the effects of interspecific interference competition on genetic diversity of *C. splendens* populations. Using amplified fragment length polymorphisms (AFLP), we determined the population genetic structure of 12 *C. splendens* populations and used the genetic diversity information to relate heterozygosity of *C. splendens* to abundance of *C. virgo* in sympatric populations. We found that heterozygosity of *C. splendens* males decreased with increasing abundance of *C. virgo* males. This result most likely reflects changes in effective population size due to interspecific interference competition and shows an effect on genetic structure in damselfly populations.

Introduction

Understanding the consequences of interspecific competition is important in both ecology and evolutionary biology. Classic ecological hypotheses suggest that two ecologically similar species struggle for co-existence, and the competition exclusion principle governs the coexistence of extremely similar species (Hardin 1960) which in most of cases are closely related (e.g. Violle *et al.* 2011). Current experimental evidence for this phylogenetically limiting similarity hypothesis coming from bacterivorous protist species, in which both frequency and tempo of competition exclusion increased with increasing phylogenetic relatedness (Violle *et al.* 2011). Alternatively, coexisting species might diverge in sympatry to reduce the cost of interspecific competition. Sympatric populations of two closely related species respond to selection by lessening resource competition, interspecific encounter rates and competitor recognition. This process is called character displacement (Brown & Wilson 1956). Interspecific competition and its consequence, character displacement, have long been viewed as an important evolutionary force making coexistence of closely related species possible. Moreover, both theoretical (Ranta

et al. 2009) and empirical studies (Viitaniemi 2009, Kahilainen *et al.* 2014) found that interspecific competition results in the reduction of effective population size and genetic diversity.

Examples of reproductive character displacement and/or agonistic character displacement come from e.g. *Calopteryx* and *Hetaerina* damselflies (Waage 1979, Tynkkynen *et al.* 2004, 2005, 2006, Andersson & Grether 2010a, 2010b 2011, Honkavaara *et al.* 2011). In those studies, character displacement in species that exhibit similar wing pigmentation traits, resulted in trait divergence in one or both species. These traits evolved to reduce costs of interspecific interactions in sympatry or with increasing relative abundance of competitive species. However, a less explored consequence of interspecific interference competition is its effects on the level of genetic diversity (Lankau & Strauss 2007, Östman 2011, Hopkins *et al.* 2012, Kahilainen *et al* 2014). Not many studies explicitly explored the consequences of interspecific interference competition, evidenced by character displacement, on genetic structure (e.g. Hopkins *et al.* 2012). Kahilainen *et al* (2014) studied the influence of interspecific interference competition on spatial genetic structure. Such studies are important as by affecting the genetic structure and diversity of populations, interference competition can thus affect the existence of a population and the whole species.

Responses to aggression are somewhat species dependent, but if aggression results in emigration, a reduction in number of successful matings or loss of suitable territory (Plaistow & Siva-Jothy 1996 , Tynkkynen *et al.* 2006), it is predicted that interspecific interference competition would lower the effective population size of the less dominant species and thus decrease the genetic diversity. Tynkkynen *et al.* (2006) showed that the time spent by the *C. splendens* males on fights with the sympatric *C. virgo* males increased with increasing abundance of the latter, which in turn could reduce male *C. splendens* mating opportunities and abilities to maintain territories.

Calopteryx damselflies are good model organisms for ecological, genetic and evolutionary studies due to their well-known ecology and behaviour (e.g. Tynkkynen *et al.* 2004,

Koskimäki *et al.* 2004; reviewed in Cordoba-Aguilar & Cordero-Rivera 2005, Suhonen *et al.* 2008). Thus, to study the influence of interspecific interference competition and aggressive behaviour on the population genetic structure, we chose two closely related damselfly species, the banded demoiselle (*Calopteryx splendens*) and the beautiful demoiselle (*C. virgo*). Males of both species establish and defend territories on a stream containing suitable vegetation patches in which females can lay eggs (Pajunen 1966, Gibbons & Pains 1992, Siva-Jothy 1999). Since the number of territories in a given habitat is limited, not all males get one (e.g. Plaistow & Siva-Jothy 1996, Tynkkynen *et al.* 2006, Rantala *et al.* 2010). Males without territories, however, will adopt non-territorial mating tactics by sneaking close or into territories of other males to secure reproductive output (*see* Córdoba-Aguilar & Cordero-Rivera 2005, Suhonen *et al.* 2008). It has been shown, however, that the territorial mating strategy is far more successful than the non-territorial one (e.g. Plaistow & Siva-Jothy 1996, Córdoba-Aguilar & Cordero-Rivera 2005).

The wing spot size in *C. splendens* is positively correlated with territory holding ability (Rantala *et al.* 2010) and negatively correlated with the amount of misdirected aggression from *C. virgo* (Tynkkynen *et al.* 2004, 2005, 2006). This heterospecific aggression affects distribution and abundance of *C. splendens* as Tynkkynen *et al.* (2006) found that after experimental removal of *C. virgo*, the number of territorial *C. splendens* males increased. Furthermore, they also found that time spent on fights increased with *C. virgo* abundance.

In this study, we examined 12 Finnish populations of *C. splendens* living in sympatry with *C. virgo* to determine their genetic diversity and structure, and the effect of the latter species abundance on those two characteristics. Tynkkynen *et al.* (2004, 2005, 2006) found that the wing spot size in *C. splendens* decreases with increasing abundance of the superior competitor *C. virgo* which has completely dark wings. Svensson *et al.* (2004) showed that in Swedish *C. splendens* populations, genetic differentiation was associated with phenotypic divergence as well as with geographic separation. We used amplified fragment length polymorphisms

Table 1. Sampling sites (location in Finland, river, coordinates), collection years and dates, and numbers of *C. splendens* males collected for genetic analysis (*n*). Abundance of *C. virgo* is the mean relative abundance of *C. virgo* for all the sampling years. Expected heterozygosity (H_e) is reported for the total number of *C. splendens* males.

Location	River	Lat. N, long. E	Number (*n*) of collected *C. splendens* males					H_e	*C. virgo* abundance (%)
			2004 (date)	2005 (date)	2006 (date)	2007 (date)	total		
Eastern	Tiekoski	62°01', 30°15'		15 (13 July)			15	0.23	59.5
Central	Janholanjoki	62°16', 26°22'			21 (7 July)		21	0.27	18.2
	Niemenjoki	62°16', 26°20'	20 (27 July)				20	0.26	23.1
	Neulajoki	62°16', 25°30'	19 (24 July)		22 (2 July)		41	0.28	22.1
	Pitkäjoki	62°07', 26°03'		20 (3 July)			20	0.22	25.0
Southern	Kylmäkoski	61°10', 23°40'	18 (9 July)	15 (5 July)			33	0.29	4.30
	Nummi	60°25', 23°56'		19 (5 July)			19	0.28	34.5
	Vantaanjoki	60°17', 24°53'	20 (4 July)	15 (4 July)			35	0.27	12.5
	Keravanjoki	60°17', 24°59'		13 (4 July)		09 (5 July)	22	0.28	19.1
	Espoonjoki	60°11', 24°37'		18 (4 July)		09 (5 July)	27	0.28	17.5
	Mankkaanjoki	60°12', 24°34'		09 (4 July)		08 (2 July)	17	0.25	36.3
	Pohja	60°06', 23°34'		21 (5 July)		15 (5 July)	36	0.29	12.9

(AFLP) (Vos *et al.* 1995) to determine genetic structure of *C. splendens* populations. We tested the prediction that genetic diversity in *C. splendens* would decrease with increasing *C. virgo* abundance since aggression from *C. virgo* would result in fewer *C. splendens* males being able to maintain territories (Tynkkynen *et al.* 2006) and mate, thereby reducing the effective population size of *C. splendens*.

Material and methods

Sampling

In July 2004–2007, using butterfly nets we caught adult males of *C. splendens* to determine their numbers at the site and for genetic analysis, and *C. virgo* to determine their numbers at the site. Sampling was carried out within a 2-m belt along 50–100 m stretch of a watercourses at 12 locations (2 to 418 km apart) in central, eastern and southern Finland (Table 1 and Fig. 1). All individuals of both species were collected during one and the same day between 10:00 and 16:00 (the peak of *C. splendens* and *C. virgo* activity; *see* Ward & Mill 2006). If the densities of males were so high that not all the males were caught, the number of uncaught males was estimated. During collection, care was taken to capture from each site both territorial and non-territorial *C. splendens* males (Tynkkynen *et al.* 2004, Honkavaara *et al.* 2011). To that end, we collected equal numbers of males from each site near the watercourse, likely inhabited by territorial males and non-territorial sneakers, and from the surrounding meadows and vegetation, likely inhabited by non-territorial, wanderer and young itenerant males. Thus, it is unlikely that our samples were biased by the excess of territorial or non-territorial males. To capture the genetic variation in the population we required at least 15 males from each *C. splendens* population (*see* e.g. Abbot *et al.* 2008). Sampling a site in consecutive years enabled capturing the natural variation in the gene pool which is expected for a species whose adults have short lifespan (~23 days; last author's unpubl. data based on field mark-recapture studies in Finland) but larval stage can last 1–2 years. At our sites, *C. splendens* is generally univolt-
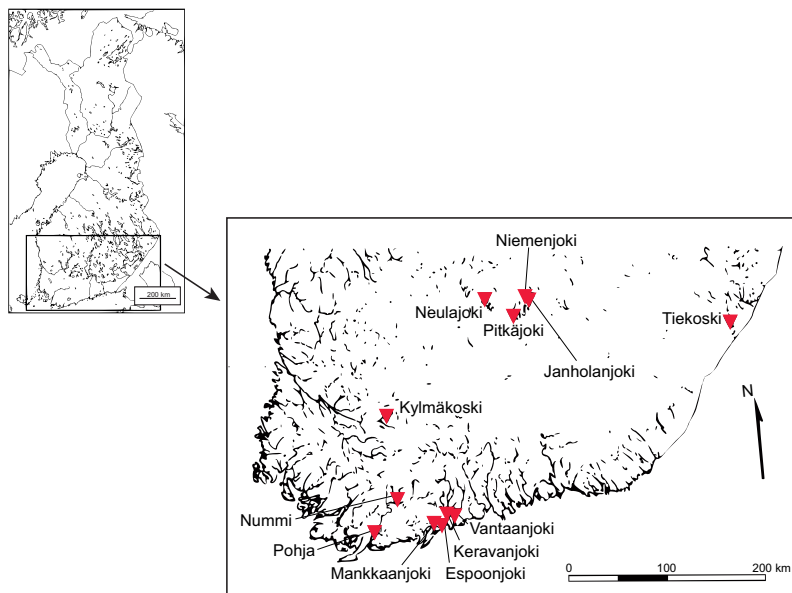
Fig. 1. Sampling area with the collection sites indicated by red triangles. For clarity, lakes are not shown.

ine, i.e. the larval stage lasts for about one year after which adults emerge (Corbet *et al.* 2006). Some adults may however emerge after two year of larval life so an overlap between different generations may be also expected which in turn can reduce the possibility of highly successful males contributing disproportionately to the gene pool in a single year. In total, we counted 410 *C. splendens* and 189 *C. virgo* males, and collected 306 *C. splendens* males which were stored in 95% ethanol before DNA analysis.

Amplified fragment length polymoprhism (AFLP) analysis

The head of each preserved *C. splendens* male was incised and placed in fresh falcon tube for DNA extraction with Qiagen DNAeasy Blood and Tissue Kit. The quality of DNA was assessed by running the DNA samples on 1% 1 × TBE-agarose gel prior to AFLP analysis. The AFLP protocol was modified according to Bensch *et al.* (2002). Restriction digests were conducted in a final volume of 15 µl containing 2.5 U EcoRI (New England Biolabs), 2.5 U MseI (New England Biolabs), 1 × Neb2 buffer (New England Biolabs), 1 µg BSA, 5 µl diluted DNA and ddH$_2$O, incubated for 3 h at 37 °C. Adapters were ligated to digested DNA by adding 4 µl of

the following mixture: 0.5 µM EcoRI adapter, 5 µM MseI adapter, 25 U T4-ligase (New England Biolabs) and 1 × ligation buffer (New England Biolabs). The reaction was incubated for 16 hours at 16° C, and diluted 8 times with 10 mM Tris-HCl.

The pre-selective PCR reaction was carried out in the final volume of 20 µl with 0.3 µM EcoRI-T, 0.3 µM MseI-C, 0.2 mM dNTPs, 2.5 mM MgCl$_2$ (Thermo Scientific), 1 × PCR buffer (Thermo Scientific), 0.4 U *Taq* polymerase (Thermo Scientific), and 10 µl diluted ligation reaction. The pre-selective PCR reaction was diluted 30 times with 10 mM Tris-HCl.

The selective PCR reaction was carried out in the final volume of 10 µl with 0.25 µM EcoRI-sel (either FAM-TAG or NED-TAC), 0.25 µM MseI-sel (either -CAC or -CCG), 0.2 mM dNTPs, 2.5 mM MgCl$_2$ (Thermo Scientific), 1 × PCR buffer (Thermo Scientific), 0.4 U *Taq* polymerase (Thermo Scientific), 2.5 µl diluted pre-selective PCR, and ddH$_2$O. Both pre- and selective PCR protocols followed Bensch *et al.* (2002). The adapters and primers used here were according to Svensson *et al.* (2004) in the following four combinations: FAM-TAG-CAC, FAM-TAG-CCG, NED-TAC-CAC, NED-TAC-CCG.

For capillary electrophoresis, 1 µl of diluted selective PCR was mixed with 10 µl of reac-

tion mixture containing 170 μl formamide (ABI) and 2 μl GS-500LIZ (ABI) size standard. The reaction was denatured for 5 min at 95 °C. The fragments created by AFLP were visualized with ABI Prism 3130xl sequencer and analysed using Genemapper ver. 4.0 (ABI). Peaks were manually checked after analysis with Genemapper. All Identified loci (137) were checked for independence of markers (Gaudeul *et al.* 2000) and non-independent loci were excluded. Analysis of a subset of 27 male heads was repeated to estimate the genotyping error rate. Loci with less than 5% variation across populations and genotyping error rate more than 10% were excluded.

Genetic structure

The final data set consisted of 78 loci for all the individuals populations (306 and 12 respectively; *see* Table 1). Euclidean distance (geographic separation) in kilometres between sampling locations was calculated using their latitude and longitude information (Table 1). It is a valid measure as the connectedness of the watersheds in our study area is relatively low. The following population genetic parameters were determined using GenAlex ver. 6.1 (Peakall & Smouse 2006): expected heterozygosity (H_e) weighted with sample size, pairwise Nei's genetic distance (Nei's D), and pairwise genetic differentiation (Φ_{PT} is an F_{ST} analogue available for binary data in GenAlex AMOVA). Principle coordinate analysis (PCoA) based on Nei's D was conducted to examine how populations group in relation to genetic distance. To test for isolation by distance, a Mantel test was conducted using PopTools ver. 3.0 with Φ_{PT} as genetic distances and their respective Euclidean distances (geographic separation), and using 999 permutations.

In addition to the Mantel test, a spatial autocorrelation analysis was conducted with GenAlex. Spatial autocorrelation allows to estimate the spatial extent of genetic structure in populations based on pairwise correlations of individual genetic similarities at set geographic separation, i.e, distance (in km) between populations (Watts *et al.* 2004, Johansson *et al.* 2005, Primmer *et al.* 2006). It was carried out by manually assigning the separation classes such

that an equal number of individual-by-individual comparisons was retained in each class. Based on the geographic distribution of the sampled populations, 11 separation classes with end points at 0, 9, 24, 39, 49, 59, 89, 119, 209, 319 and 419 km were defined. In the analysis, The classes were considered separate meaning that each individual pair belonged to only one class.

To account for habitat characteristics, using Spearman's correlation we tested for dependence between stream length and heterozygosity. Stream length was the length of a watercourse between the up- and downstream lakes it connected. We chose Spearman's correlation because of a small effective population size which usually negatively affects heterozygosity.

Interspecific interaction

According to Tynkkynen *et al.* (2004, 2005, 2006), the relative abundance of *C. virgo* males is a reliable indicator of the level of interspecific aggression towards *C. splendens* males. To calculate the relative abundance of *C. virgo* males, the total number of *C. virgo* males was divided by the total number of males of both species. Because the *C. virgo* numbers were observed to vary slightly between years (Tynkkynen *et al.* 2004), we calculated the weighted mean relative abundance of *C. virgo* males for the sampling years. Relative abundance was weighted with each year's sample size.

For statistical testing, relative abundance of *C. virgo* was arcsine-square-root transformed to allow for testing with parametric procedures. The association between the relative abundance of *C. virgo* and the heterozygosity in *C. splendens* was tested with Pearson's correlation. All statistical tests were performed with PASW ver. 18 for Windows.

Results

Genetic structure

The mean expected heterozygosity (H_e) was 0.27 (SE = 0.006), ranging from 0.22 to 0.29 (Table 1). We found no effect of the stream
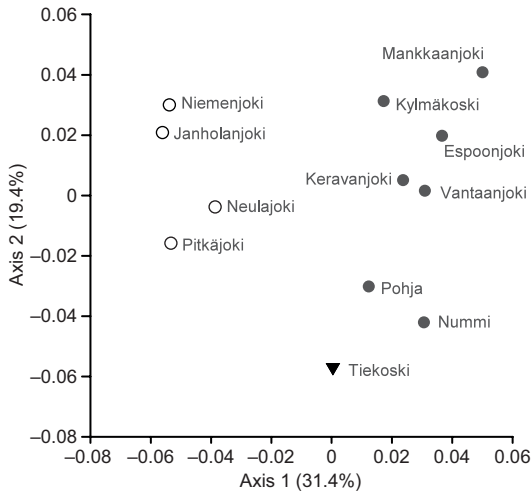
Fig. 2. Principal coordinate analysis based on Nei's genetic distance (*D*) between *C. splendens* populations; dot = southern Finland, circle = central Finland and triangle eastern Finland.

length or *C. splendens* population size on heterozygosity (Spearman's correlation: $r_S = 0.11$, $n = 12$, $p = 0.73$; and $r_S = –0.23$, $n = 12$, $p = 0.475$; respectively).

In PCoA based on Nei's *D* (Fig. 2), Axis 1 separates the populations according to their geographical location. Although, the most eastern population, Tiekoski, was at the same latitude as the populations from central Finland, in PCoA it grouped between the central and southern populations (Fig. 2).

AMOVA proved a significant genetic differentiation between the populations. The mean

Φ_{PT} was 0.08 ($p = 0.001$; 1000 permutations) ranging from 0 to 0.14 (Table 2). Only two populations, Janholanjoki and Niemenjoki, both in the same catchment area and separated only by a small lake, were identical ($\Phi_{PT} = 0$). The eastern Tiekoski population was found to be genetically closer to populations from southern Finland (Table 2 and Fig. 2).

Mantel's test revealed that population isolation by distance (geographic separation) was significant ($r = 0.40$, $n = 66$, $p < 0.001$; Fig. 3). Spatial autocorrelation analysis results supported the above: individuals separated by less that 50 km are genetically more similar, and those separated by more than 150 km are genetically different (Fig. 4).

Interspecific interaction

The relative abundance of *C. virgo* varied between 4.3% and 59.5% in sampled *C. splendens* populations (Table 1), with the values between 12% and 25% being most common. The heterozygosity in *C. splendens* populations decreased with increasing relative abundance of *C. virgo* (Pearson's correlation: $r = –0.62$, $n = 12$, $p = 0.031$) (Fig. 5).

Discussion

Mean population expected heterozygosity ($H_e = 0.27$) was similar to that found for damselflies

Table 2. Geographic separation (km, upper diagonal) and pairwise genetic distance (Φ_{PT}) values (from AMOVA, lower diagonal) between *C. splendens* populations. Significant Φ_{PT} values ($p < 0.05$) are set in boldface.

	1	2	3	4	5	6	7	8	9	10	11	12
1. Nummi	–	53	220	40	222	242	243	84	41	381	44	59
2. Vantaanjoki	**0.04**	–	212	76	221	232	233	116	20	345	19	6
3. Pitkäjoki	**0.14**	**0.11**	–	261	33	22	23	164	227	219	227	212
4. Pohja	**0.05**	**0.06**	**0.10**	–	262	283	283	117	57	417	59	81
5. Neulajoki	**0.12**	**0.12**	**0.09**	**0.12**	–	43	44	156	234	248	235	222
6. Niemenjoki	**0.09**	**0.09**	**0.06**	**0.10**	**0.09**	–	2	186	248	206	248	232
7. Janholanjoki	**0.09**	**0.09**	**0.07**	**0.09**	**0.09**	0	–	187	248	204	249	232
8. Kylmäkoski	**0.05**	**0.02**	**0.11**	**0.06**	**0.09**	**0.06**	**0.07**	–	117	360	119	121
9. Mankkaanjoki	**0.07**	**0.04**	**0.14**	**0.09**	**0.12**	**0.11**	**0.10**	**0.05**	–	365	3	25
10. Tiekoskinjoki	**0.06**	**0.05**	**0.11**	**0.07**	**0.08**	**0.11**	**0.10**	**0.06**	**0.10**	–	364	341
11. Espoo	**0.04**	**0.03**	**0.13**	**0.08**	**0.11**	**0.10**	**0.10**	**0.03**	**0.06**	**0.06**	–	23
12. Keravanjoki	**0.04**	**0.02**	**0.12**	**0.07**	**0.12**	**0.09**	**0.10**	**0.04**	**0.03**	**0.07**	**0.04**	

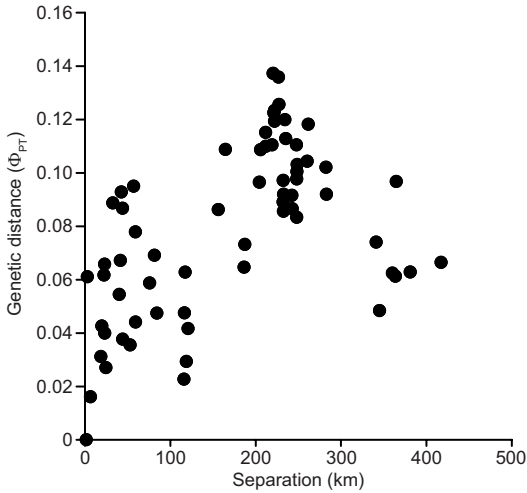**Fig. 3.** Genetic isolation by distance of 12 *C. splendens* populations in Finland according to Mantel's test ($r =$ 0.40, $n = 66$, $p < 0.001$).
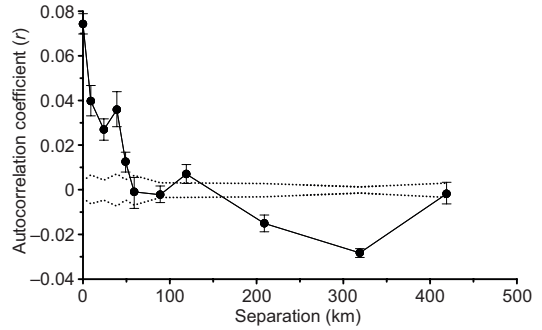

Fig. 4. Spatial genetic structure of *C. splendens* populationsa as revealed by spatial autocorrelation; r (solid lines and dots) represents pairwise correlation of genetic similarities between individuals belonging to the same separation class, error bars are the 95% confidence intervals for r in each separation class determined by bootstrapping. The dotted lines show the upper and lower 95% confidence limits for r assuming no spatial autocorrelation as determined by bootstrapping.

in earlier studies (e.g. Svensson *et al.* 2004, Chaput-Bardy *et al.* 2008, Sato *et al.* 2008, Abbott *et al.* 2008, Kahilainen *et al.* 2014), and H_e decreased with increasing relative abundance of *C. virgo* which is in line with the result of Kahilainen *et al.* (2014).

According to Eckert *et al.* (2008), populations at the edges of a species distribution limits are characterised by lowered expected heterozygosity may (e.g. Eckert *et al.* 2008). This, however, was not the case in our study, as *C. splendens* populations near the species distribution limits (Wellenreuther *et al.* 2012) had much higher levels of heterozygosity (Table 1).

Kahilainen *et al.* (2014) found more variation in heterozygosity than in allelic richness in *C. splendens* and suggested generally low genetic diversity as evidence of the range-margin effect. It is, however, unlikely that in our study, decreased heterozygosity was the effect of anything else than interspecific competition as, according to our results, habitat size and/or population size had no effect on heterozygosity. Kahilainen *et al.* (2014) also reported that in their study water temperature did not differ between *C. splendens* and *C. virgo* habitats: as both species prefer similar habitats, habitat conditions should not have affected heterozygosity at the sites we sampled. Thus, in both studies, abundance of *C. virgo* living in sympatry with *C.*
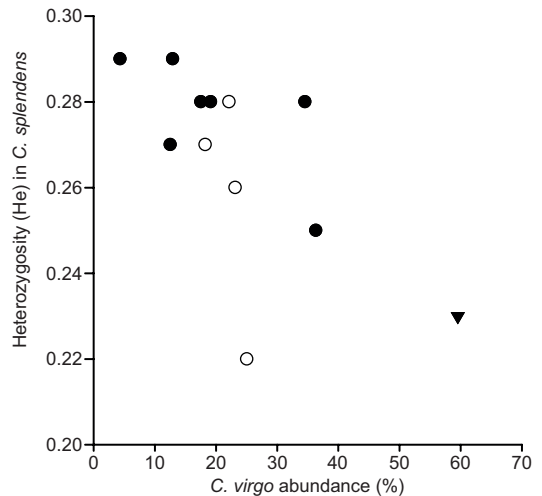

Fig. 5. Heterozygosity of *C. splendens* in relation to weighted over the sampled years mean abundance of *C. virgo* (%) in the 12 populations in Finland (Pearson's correlation: $r = -0.62$, $n = 12$, $p = 0.031$). Symbols denote the collection sites in Finland: dot = southern Finland, circle = central Finland and triangle eastern Finland.

splendens better explains the observed decreased heterozygosity in *C. splendens*. In other words, the interaction between the two species affects the genetic structure of *C. splendens* through different mechanisms affecting e.g. reproduction such as interspecific aggression (Tynkky-

nen *et al*. 2004, 2006, Svensson *et al*. 2007). It is also possible that the level of parasitism *C. splendens* increases when in sympatry with *C. virgo* (Ilvonen *et al*. 2011). Additionally, it may be difficult for *C. splendens* immigrants to acquire territories in areas occupied by *C. virgo* or immigration may not be possible altogether (Kahilainen *et al*. 2014).

In our study, the only genetically similar *C. splendens* populations were those from Janholanjoki and Niemenjoki (Table 1). In previous mark-recapture *C. splendens* studies it was found that males can either fly over one kilometre (e.g. Stettmer 1996) or be sedentary (Tynkkynen *et al*. 2005, Rantala *et al*. 2010).

The *C. splendens* genetic distance (Φ_{PT}) values in our study were similar to those found for damselfly in previous studies (Svensson *et al*. 2004, Chaput-Bardy *et al*. 2008, Sato *et al*. 2008, Abbott *et al*. 2008, Kahilainen *et al*. 2014). In their study, Kahilainen *et al*. (2014) found no evidence for isolation by distance even though their geographic sampling range was wider than ours and the overall sampling scheme was similar. They, however, used different type of genetic markers than those in our study. AFLP provides simple presence/absence whereas microsatellites allow multiple alleles per marker. Without genome sequence information it is though difficult to estimate whether genomic location of the markers could have affected the results.

In *C. splendens* populations, males with territories mate most successfully (Plaistow & Siva-Jothy 1996). When in sympatry with *C. virgo*, the number of *C. splendens* males with territories increases when the abundance of *C. virgo* males is lowered by manipulation (Tynkkynen *et al*. 2006). Presence of parasites could also play a role in reducing genetic diversity in *C. splendens* populations sympatric with *C. virgo*. Eugregarine trophoizoites, an intestine parasite, reduces the reproductive success of males as infected individuals are unable maintain to territories as effectively as the healthy ones (Siva-Jothy & Plaistow 1999, Marden & Cobb 2004; also reviewed in Córdoba-Aguilar & Cordero-Rivera 2005). Ilvonen *et al*. (2011) found that prevalence of gregarine parasites in *C. splendens* is higher when in sympatry with *C. virgo*, and Kaunisto *et al*. (2013) that *C. splendens*

males with high number of eugarine parasites had lower genetic diversity than non-parasitized males. Consequently, *C. splendens* populations may face a higher extinction risk when occupying the same sites as *C. virgo* due to interference competition reducing genetic diversity and reproduction output (Ranta *et al*. 2009), as well as due to increased risk of parasite infestation causing mortality. In addition, harassment of *C. splendens* females by *C. virgo* males can result in lower female fecundity or even death. This has been shown in *Hetaerina* damselflies, in which females harassed by males produced only one batch of eggs instead of several ones (Córdoba-Aguilar 2009, Córdoba-Aguilar & González-Tokman 2011).

It is possible that a combination of factors leads to the decrease in heterozygosity of *C. splendens* in the presence of *C. virgo*. The time *C. splendens* males spend on fighting with *C. virgo* males is the time taken away from courtship and mating (Tynkkynen *et al*. 2006), which can lead to a lower number of males reproducing in a given year and thus reduce the effective population size. Also, to avoid competition for territories with *C. virgo*, large spotted *C. splendens* males are likely to acquire less optimal territories further away from those of the former (Tynkkynen *et al*. 2006). Since habitat characteristics are important for successful reproduction of *C. splendens* (e.g. Gibbons & Pain 1992), the number of successful ovipositions might decrease due to lower quality of the habitat. This could lead to reduction in population size in the following years, which in turn has been shown to negatively affect genetic diversity (Obrien *et al*. 1983, Menotti-Raymond & Obrien 1993). This may also be a reason for decreased *C. splendens* heterozygosity found in our study.

According to Kokko and Brooks (2003), a small population with minimal genetic variation may not be able to withstand changes in environmental conditions or respond to parasites as successfully as a highly genetically diverse large population. On the other hand, sexual selection may affect the chances of population extinction. Inbreeding can either increase or decrease population viability in sexual selection. It may be caused by high mating skew as in *Calopteryx* males (Plaistow & Siva-Jothy 1996), but it could

be reduced if females adaptively chose mates to avoid inbred offspring. There is empirical evidence that mating success is positively correlated with genetic quality within population (e.g. Møller & Alatalo 1999).

Another explanation for the decline in heterozygosity of *C. splendens* due to interspecific competition involves the intraspecific dynamic between *C. splendens* males. In sympatry, selection for divergence in male signalling traits (Tynkkynen *et al.* 2004, 2005, 2006, Svensson *et al.* 2006) is important since hybridization with *C. virgo* is maladaptive (Tynkkynen *et al.* 2008). Since *C. splendens* males with large wing spots tend to obtain the best territories in the absence of *C. virgo*, they could be viewed as the "best quality" mates. If the "best quality" males are displaced due to interspecific competition with *C. virgo* males, then more of the "lower quality"(i.e. with small wing spot) males will be able to mate and contribute to the population. Furthermore, if there is an association between male quality and heterozygosity in *C. splendens* similar to that found in other species (e.g. Höglund *et al.* 2002, Marshall *et al.* 2003), it would follow that the heterozygosity in the *C. splendens* population would decrease. Svensson *et al.* (2004) showed a significant correlation between phenotypic diversity and genetic diversity at the population level; however it should also be examined at individual level to see if the phenotype is a reliable indicator of genetic diversity, or heterozygosity, in *C. splendens* males.

We studied only the populations of *C. splendens* living with sympatry with *C. virgo*, thus genetic information from allopatric populations could give more insight into the population structure of the species. As Svensson *et al*. (2006) showed, sexual selection can have different trajectories in sympatric and allopatric populations. There is cyclicity in population size, as well as variation in larval development time causing variation in the gene pool in a given year. We are aware of the limitations in our study stemming from small sample size and sampling in a single day, but as mark–recapture based studies (Tynkkynen *et al*. 2006, Rantala *et al*. 2010) show, numbers of males can be estimated quite reliably this way. Future studies should focus on the role of interspecific competition as well as other forms of interspecific interactions that may affect genetic diversity. In the case of *C. splendens* it is likely that there are several factors influencing effective population size and thus the genetic diversity of a population.

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