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ORIGINAL PAPER

Fine-scale spatial genetic structure and within population male-biased gene-flow in the grasshopper *Mioscirtus wagneri*

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Abstract Dispersal is a life history trait that plays a key role in population dynamics, determining gene flow and influencing the size, structure and persistence of populations. For these reasons, the study of the genetic consequences of dispersal can be considered a central topic in both conservation and population genetics. In this study we examine the patterns of fine-scale genetic structure within two populations of the grasshopper *Mioscirtus wagneri* (Orhoptera: Acrididae). For this purpose, we have used seven species-specific microsatellite markers to type 266 individuals from two populations (Peña Hueca and El Salobral) located in Central Spain. We have found subtle genetic differentiation between some sampling patches and significant kinship structures up to 25 m distance which were particularly patent for females. In Peña Hueca locality, patterns of isolation-by-distance at both the patch scale and the individual level have also revealed an association between genetic differentiation/similarity and geographical distance in females but not in males. Overall, these data suggest a fine-scale spatial genetic substructure in the studied populations which seems to be mainly driven by female philopatry. Such pattern of within population genetic structure together with the inferred restricted dispersal distances is likely to contribute to reduce effective population sizes and inter-population gene flow. This can erode genetic variability and limit the colonization ability of this orthoptera, factors which can ultimately compromise the long-term persistence of their small size and isolated populations.

Keywords Genetic structure · Isolation by distance · Microsatellites · *Mioscirtus wagneri* · Sex-biased dispersal

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Introduction

Dispersal is a life history trait that plays a key role in population dynamics, determining gene flow and influencing the size, structure and persistence of populations (Dieckmann et al. 1999; Clobert et al. 2001). This behaviour greatly contributes to determine the distribution of genetic variability in natural populations so that the study of dispersal can be considered a central issue in population genetics (Clobert et al. 2001). Data on dispersal rates and patterns are not only important to understand inter-population gene flow and structure but also to comprehend the dynamics within apparently homogeneous populations (Sugg et al. 1996; e.g. Coltman et al. 2003; Nussey et al. 2005; Ortego et al. 2008a, b; Galarza et al. 2009). Restricted dispersal can result in genetic differentiation at local scales and favour the maintenance of fine-spatial scale selection processes and adaptations (Coltman et al. 2003; Coltman 2005; Garant et al. 2005; Postma and van Noordwijk 2005). Low dispersal rates can also diminish effective population sizes, resulting in reduced within population genetic variability due to a combination of inbreeding and random genetic drift (Frankham 1996; Sugg et al. 1996; Ortego et al. 2008a). This is relevant, because the level of genetic variability within a population can greatly determine its long-term maintenance and viability (Saccheri et al. 1998; Frankham 2005; Willi et al. 2006).

The asymmetric dispersal of sexes and its ecological, evolutionary and genetic consequences have also received much attention by biologists (Clobert et al. 2001). In general, male biased dispersal is the norm in mammals, whereas the reverse pattern is generally found in birds (Greenwood 1980; Dobson 1982; Clarke et al. 1997). Three main general hypotheses have been proposed to explain sex biased dispersal patterns under different mating systems or life history characteristics: competition for resources (Greenwood 1980), competition for mates (Dobson 1982; Perrin and Mazalov 2000), and avoidance of inbreeding (Pusey 1987; Perrin and Mazalov 2000). Despite the study of dispersal patterns is a major topic in ecology and evolutionary biology, scarce information is available on several other organisms groups in which long-term or detailed monitoring of individuals is particularly complicated (Knight et al. 1999). Although examples on sex-biased dispersal in animals other than birds and mammals are also growing (e.g. Goodisman and Ross 1998; Gyllenstrand and Seppa 2003; Clemencet et al. 2005; Bailey et al. 2007; Suni and Gordon 2010), information for certain taxa is still rare and additional research would allow to test genuine predictions about dispersal bias in scarcely explored taxonomic groups (Goudet et al. 2002).

The advent and application of DNA polymorphic markers together with novel statistical procedures have provided new methods to obtain indirect estimates of dispersal and fine-scale spatial genetic structure in natural populations (Prugnolle and de Meeus 2002; Hardy and Vekemans 2002; Jensen et al. 2005; Peakall and Smouse 2006; Broquet and Petit 2009). In general, most of these methods are based on the fact that species/populations with limited dispersal are characterized by a pattern of increased genetic differentiation with geographical distance in comparison with those showing unrestricted dispersal (Rousset 2000; Prugnolle and de Meeus 2002; Vitalis 2002; e.g. Knight et al. 1999; Temple et al. 2006; Watts et al. 2007). Thus, limited dispersal results in close spatial associations between relatives which is reflected by fine-spatial scale genetic structure (Nussey et al. 2005). A key advantage of new analytical tools is that they are often based on individuals rather than on populations or subpopulations, allowing more accurate estimates of contemporary patterns of dispersal at local spatial scales (Manel et al. 2003). The potential of these molecular approaches to analyze sex-biased dispersal has also received much attention in recent years (Goudet et al. 2002; Prugnolle and de Meeus 2002; Vitalis 2002),

although the general ability of these methods to reveal such asymmetric patterns has been criticised in some studies (Rassmann et al. 1997; Goudet et al. 2002; Hansson et al. 2003; Vitalis 2002).

The objective of this study is analysing the patterns of dispersal and fine-scale genetic structure within two populations of the grasshopper *Mioscirtus wagneri* (Kittary 1859) (Orthoptera: Acrididae). *M. wagneri* is a highly specialist grasshopper exclusively inhabiting hypersaline low grounds with patches of *Suaeda vera*, the halophilic plant on which it exclusively depends for food (Cordero et al. 2007; Ortego et al. 2010). Previous recent studies have revealed strong genetic structure at different geographical scales, probably due to the isolation and patchy distribution of the particular habitats required by this species (Ortego et al. 2009; Ortego et al. 2010). However, the pattern of fine-scale spatial genetic structure has been never analyzed despite this information is crucial to understand contemporary within-population dispersal patterns (Ortego et al. 2010). The previous studies at a large geographical scale can only provide partial information on contemporary patterns of dispersal and gene flow because most analyzed populations seem to mostly behave as discrete and isolated populations with rare dispersal events among them (Ortego et al. 2010). The relative dispersal of each sex has been rarely studied in orthoptera (Lorch and Gwynne 2000; Bailey et al. 2007), probably due to the difficulty of monitoring marked individuals (e.g. Riegert et al. 1954; Lorch et al. 2005) together with the lack of suitable nuclear markers necessary to obtain detailed information on fine-scale spatial genetic structure (Ustinova et al. 2006; Chapuis et al. 2008). The single-year generation time of *M. wagneri* makes this species an interesting model system to study asymmetric dispersal of sexes using molecular methods. In contrast with long-lived organisms, the patterns of contemporary sex-biased dispersal are likely to be accurately estimated in *M. wagneri* because parental alleles will never co-occur in the sampling population with those transmitted to their offspring (Goudet et al. 2002; Vitalis 2002; Hansson et al. 2003). Further, this species shows a pronounced reversed sexual size dimorphism (females being $\sim$ 30 % larger than males; Cordero et al. 2007), suggesting higher movement capability and dispersal potential of females (e.g. Palo et al. 2004).

We have used seven species-specific microsatellite markers to analyze the patterns of fine-scale genetic structure and infer the dispersal behaviour within the studied populations of the grasshopper *M. wagneri*. In particular, we tested the following predictions: (1) due to small body size and the particular habitat requirements of this species, we would expect restricted dispersal and significant genetic differentiation among sampling patches within the studied local spatial scale (< 2.5 km); (2) we also predicted that both genetic differentiation between sampling patches and genetic distance between individuals increase with increasing geographical distance (i.e. isolation by distance) due to migration-drift equilibrium; (3) finally, due to the pronounced reversed sexual size dimorphism in this species we expected a higher dispersal capability of females. Accordingly, this would have resulted in (3.1) a stronger genetic structure (3.2) a higher local genetic relatedness, and (3.3) a more marked isolation-by-distance pattern of genetic structure in males than in females.

Materials and methods

Sampling and study area

We studied patterns of gene flow within two populations (Peña Hueca and El Salobral) of *M. wagneri* located in Toledo province, Central Spain (Fig. 1). Peña Hueca, a stationary

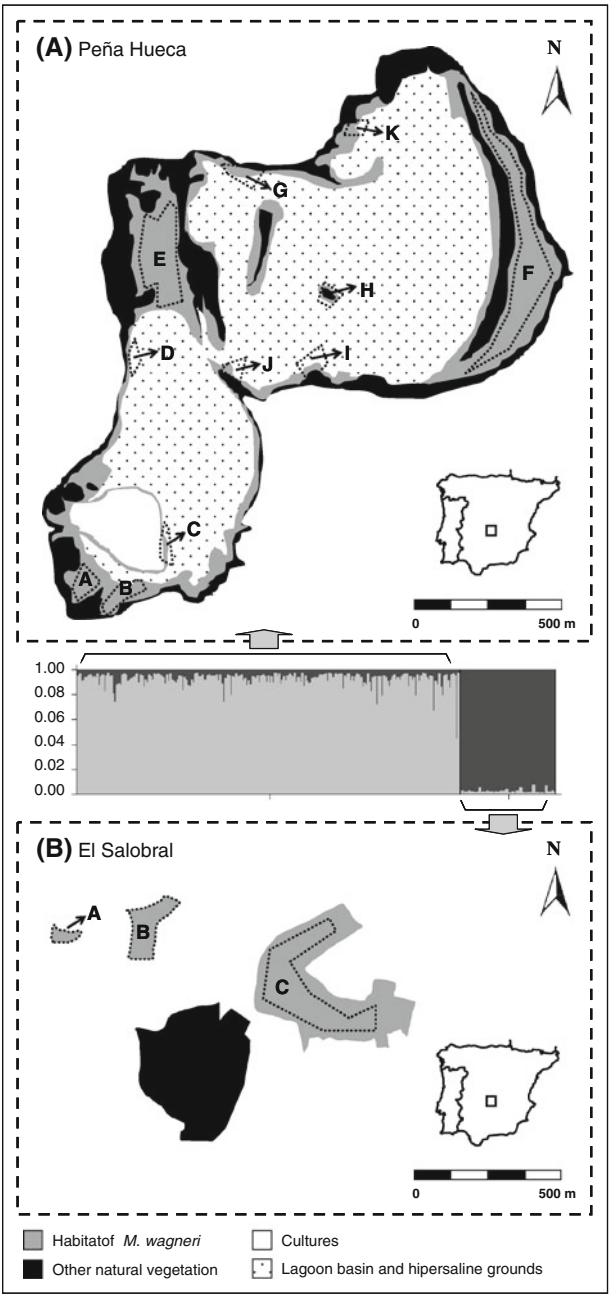

Fig. 1 Maps of the studied localities showing habitats of *Mioscirtus wagneri* and other land covers. *Dotted polygons* delimitate the sampling plots in each locality. The genetic assignment based on Bayesian method implemented in the program STRUCTURE is also showed. Each individual is represented by a thin *vertical line*, which is partitioned into two coloured segments that represent the individual's probability of belonging to the cluster with that *colour*

hypersaline lagoon with strong summer drought, and surrounding natural habitats constitute a protected natural reserve (39°31′ N, 3°20′ W; Fig. 1a). In this locality *M. wagneri* occupies the vegetation ring with *S. vera*, the halophilic plant on which it exclusively depends for food (Cordero et al. 2007). El Salobral is a saline low ground with three patches of *S. vera* located within a matrix of cereal cultures (39°38′ N, 3°13′ W; Fig. 1b). During 2009 (Peña Hueca: July 14th; El Salobral: July 21st), we collected 212 adult individuals (118 males and 94 females) in Peña Hueca locality and 54 adult individuals in El Salobral population (32 males and 22 females). Sampling took place during the species reproductive season, when all individuals are adults and show an active reproductive behaviour (including sexual displays and copulation; P. J. Cordero, unpublished data). We extensively sampled 11 and 3 patches covering the entire distribution range of *M. wagneri* within Peña Hueca and El Salobral populations, respectively (Fig. 1). Densities of *M. wagneri* sharply decline at low covers of *S. vera* and it virtually disappears few meters beyond the patches of this plant (Cordero et al. 2007). For this reason, we considered as sampling patch any area with a continuous cover of *S. vera* and areas where this plant species was absent were considered as habitat discontinuities to delimit sampling patches. We aimed to sample a similar number of males and females in each patch. However, male to female ratio is generally much higher than parity in this species (P. J. Cordero, unpublished data) and this generally resulted in smaller sample sizes for females in most sampling patches (Table 1). Sample size in each patch varied according with patch size and local population densities. We sampled individuals covering the entire patch area and the spatial coordinates of each specimen were registered using a Global Positioning System (GPS). Specimens were preserved whole in 1,500 µl ethanol 96% at -20°C until needed for genetic analyses. Further information on the study populations and sampled patches is given in Fig. 1 and Table 1.

Table 1 Sample sizes and estimates of genetic variability for the patches sampled in each studied population

Patch	*N* total	*N* males	*N* females	A_R	A_{Priv}	F_{IS}
(a) Peña Hueca population						
PEN-A	17	10	7	4.86	0.07	0.097
PEN-B	22	7	15	5.33	0.29	0.093
PEN-C	10	7	3	4.97	0.25	−0.106
PEN-D	15	8	7	5.05	0.24	0.157
PEN-E	30	18	12	4.92	0.10	0.078
PEN-F	28	18	10	5.24	0.23	0.034
PEN-G	15	8	7	4.93	0.11	0.047
PEN-H	14	11	3	4.94	0.13	−0.015
PEN-I	27	11	16	5.16	0.21	0.003
PEN-J	17	10	7	5.25	0.18	0.068
PEN-K	17	10	7	5.56	0.08	0.054
(b) El Salobral population						
ELS-A	20	10	10	4.68	0.13	−0.033
ELS-B	18	12	6	4.75	0.17	−0.033
ELS-C	16	10	6	4.81	0.12	0.002

A_R mean standardized allelic richness, A_{Priv} mean number of private alleles, F_{IS} inbreeding coefficient

Microsatellite genotyping

We genotyped individuals using seven polymorphic microsatellite markers isolated and characterized from a genomic library of a *M. wagneri* specimen (MwGTC8, MwGTD9, MwGTG12, MwGTA6, MwGTC12, MwGTC11, MwGATAB 11; Aguirre et al. 2010). We used NucleoSpin Tissue (Macherey-Nagel, Düren, Germany) kits to extract and purify genomic DNA from a hind leg of each individual. Amplifications were conducted in 10-µl reaction volumes containing 5 ng of template DNA, 1× reaction buffer (67 mM Tris–HCL, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01 % Tween-20, EcoStart Reaction Buffer, Ecogen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 µM of each dye-labelled primer (FAM, PET, NED or VIC) and 0.1 U of *Taq* DNA EcoStart Polymerase (Ecogen). All reactions were carried out on a Mastercycler EpgradientS (Eppendorf) thermal cycler. The PCR programme used was 9 min denaturing at 95°C followed by 35 cycles of 30 s at 94°C, 45 s at the annealing temperature (Aguirre et al. 2010) and 45 s at 72°C, ending with a 5 min final elongation stage at 72°C. Amplification products were run on an ABI 310 Genetic Analyzer (Applied Biosystems) and genotypes were scored using GeneMapper 3.7 (Applied Biosystems).

Genetic diversity estimates

Microsatellite genotypes were tested for departure from Hardy-Weinberg equilibrium at each locus using an exact test (Guo and Thompson 1992) based on 900 000 Markov chain iterations as implemented in the program Arlequin 3.1 (Excoffier et al. 2005). We also used Arlequin 3.1 to test for linkage equilibrium within each pair of loci using a likelihood-ratio statistic, whose distribution was obtained by a permutation procedure (Excoffier et al. 2005). Measures of allelic richness (A_R) and number of private alleles (A_{Priv}) for each sampling patch were standardized for sample size using the program HP-Rare (Kalinowski 2005). Inbreeding coefficients (F_{IS}) were calculated following Nei (1977).

Genetic structure between the studied populations

We analyzed the spatial genetic structure between the studied populations (Peña Hueca and El Salobral) using an individual-based approach as implemented in the program Structure (version 2.3.3; Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009). The program Structure 2.3.3 is a Bayesian model-based clustering method which assigns individuals to populations based on their multilocus genotypes (Pritchard et al. 2000; Falush et al. 2003). For K population clusters, the program estimates the probability of the data [Pr($X|K$)] and the probability of individual membership in each cluster using a Markov chain Monte Carlo (MCMC) method. We ran Structure assuming correlated allele frequencies and admixture (Pritchard et al. 2000; Falush et al. 2003) and using prior population information (Hubisz et al. 2009). We conducted five independent runs for each value of K to estimate the true number of clusters with 10^6 MCMC cycles, following a burn-in period of 100 000 iterations. The simulated values of K ranged from 1-4. The number of populations best fitting the data set was defined both using log probabilities [Pr($X|K$)] and ΔK, as described in Evanno et al. (2005). Thus, the estimated number of subpopulations is taken to be the value of K at which Pr($X|K$) plateaus.

Within population genetic structure

We investigated population genetic structure within the studied populations of *M. wagneri*. For this purpose we calculated pairwise F_{ST} values between sampling patches and tested

their significance with Fisher's exact tests after 10 000 permutations as implemented in ARLEQUIN 3.1 (Excoffier et al. 2005). We also calculated overall genetic differentiation using the software PCAGEN (www.unil.ch/izea/softwares/pcagen.html) with 10 000 randomization steps. We analyzed all sampled individuals together and male and female genotypes separately. Only patches with six or more sampled individuals were considered in female analyses (Table 1). We used two methods to analyze differences in gene flow (F_{ST}) between males and females: 1) First, we tested whether there was a significant difference in average pair-wise F_{ST} between males and females (Bailey et al. 2007); 2) Second, we used the randomization method implemented in FSTAT version 2.9.3 (10,000 permutations) to analyze differences between sexes in inter-patch genetic differentiation (F_{ST}) (Goudet 2001; Goudet et al. 2002).

The spatial genetic structure of the study population was also tested using multilocus spatial correlation analyses in GENALEX version 6.0 (Peakall and Smouse 2006). This software calculates an autocorrelation coefficient r using two pairwise matrices, one containing geographic distances and the other containing squared genetic distances. The autocorrelation coefficient r is calculated for a specified number of distance classes, and provides a measure of the genetic similarity between pairs of individuals falling within each distance class. Pairwise squared genetic distances were obtained one locus at a time using the methods described in Peakall et al. (1995) and Smouse and Peakall (1999). Genetic distances for each locus are summed across all loci to obtain covariance matrices, under the assumption of statistical independence. This assumption is reasonable, since the seven microsatellites employed showed no evidence of linkage disequilibrium (Aguirre et al. 2010). The linear pairwise geographic distance matrix was calculated from the x- and y-coordinates obtained using a Global Positioning System (GPS) for each captured individual. Variable distance classes were used because previous research suggested that spatial autocorrelation was likely to occur only in the first distance classes (e.g. Double et al. 2005; Ortego et al. 2008b). Distance classes were set at 25 m, 50 m, 100 m, 200 m, 400 m, 800 m, and 1,600 m. The same distance classes were used to analyze all sampled individuals together and male and female genotypes separately. The calculated autocorrelation coefficients r, were plotted as a function of distance to produce spatial genetic autocorrelograms. Tests for statistical significance were performed using two methods: random permutation and bootstrap estimates of r, with the number of permutations and bootstraps set to 999 (Peakall et al. 2003).

Isolation by distance

We explored the occurrence of an isolation-by-distance pattern of spatial genetic structure using two different approaches:

(i) We compared pairwise matrices of genetic (F_{ST}) and Euclidean geographical distances between sampling patches. For this purpose, we used IBDWS version 3.16, which performs a Mantel test and a Reduced Major Axis (RMA) regression analysis (Jensen et al. 2005). The significance of Mantel test was assessed by 10 000 randomizations of the genetic distance matrix. The test was one-tailed as only a positive correlation between geographical and genetic distances is expected (e.g. Gómez et al. 2007; Ortego et al. 2010). This approach was used to analyze the entire dataset and male and female data separately. We tested whether there was a significant difference between males and females in the slope values from linear regression analyses between pair-wise F_{ST} and Euclidean geographic distances performed for each analyzed patch separately (Bailey

et al. 2007). This approach was only used to analyze data for Peña Hueca population. The small number of patches in El Salobral population ($n = 3$ patches) precluded the possibility of performing this patch-based analysis in this locality.

(ii) We compared the slopes of all individual regressions of pairwise relatedness on geographical distance (Knight et al. 1999; Stow et al. 2001; Hazlitt et al. 2004). For this purpose, we first calculated for each individual its relatedness with all other sampled individuals. We used the software MARK (K. Ritland; www.genetics.forestry.ubc.ca/ritland/programs.html) to calculate pairwise relatedness values for all individuals using Queller and Goodnight's (1989) estimator. On the other hand, we calculated the pairwise geographic distance between all individuals using an EXCEL spreadsheet. We then performed a separate regression of relatedness on pairwise distance for each individual, and obtained the slope value (b) of this regression. A negative b value indicates that genetic similarity decreases with distance, suggesting an "isolation-by-distance" pattern of genetic structure (Slatkin 1993). We repeated this process for each individual, and then we determined whether the b values obtained for all individuals differed significantly from zero using a Student's t-test. We also used a general linear model (GLM) to analyze whether the slope values differed between sexes. In this analysis we also included patch identity as fixed factor to control for possible differences between patches in individual genetic relatedness which may arise due to differential dispersal or immigration (Table 2). Note that the obtained slope values (b) did not differ from a normal distribution (Kolmogorov-Smirnov test; $Z = 0.396$; $P = 0.998$). This approach was used to analyze all sampled individuals and only considering male-male and female-female comparisons (Knight et al. 1999; Stow et al. 2001; Hazlitt et al. 2004; see also Ortego et al. 2008b). These individual-based analyses were performed in both studied populations.

Results

Genetic diversity

We found no evidence of linkage disequilibrium among loci, indicating that the analyzed markers can be treated as independent from each other. After adjusting for multiple comparisons, one locus (GTA6) significantly deviated from HWE due to heterozygote deficiency. A total of 87 alleles were observed across all populations of *M. wagneri* over the seven analyzed loci. A_R estimated considering all loci ranged from 4.68 to 5.56 alleles in ELS-A and PEN-K sampling patches, respectively (Table 1). Eight out of 14 sampling patches exhibited private alleles (Table 1; Supplementary Table 1).

Population genetic structure

STRUCTURE analyses revealed a maximum $\Pr(X|K)$ for $K = 2$, indicating the presence of two genetic clusters corresponding with the two studied populations (Fig. 1). Pairwise F_{ST} values indicated significant levels of genetic differentiation between some sampling patches. After sequential Bonferroni correction, we found two pairwise significant F_{ST} values for female genotypes in Peña Hueca population, whereas all pairwise comparisons resulted significant in El Salobral (Table 2). However, no F_{ST} value remained significant for male genotypes in any studied population (Table 2), suggesting a lower genetic structure in

Table 2 Pairwise population F_{ST} values between sampling patches only considering males (above the diagonal) and females (below the diagonal)

Patches	PEN-A	PEN-B	PEN-C	PEN-D	PEN-E	PEN-F	PEN-G	PEN-H	PEN-I	PEN-J	PEN-K
(a) Peña Hueca locality											
PEN-A	–	0.009	0.003	0.003	−0.014	0.027	−0.012	−0.006	0.012	0.012	0.019
PEN-B	0.050	–	−0.034	−0.016	−0.002	−0.015	−0.004	0.001	−0.018	−0.008	0.003
PEN-C	–	–	–	−0.009	0.013	−0.004	0.007	−0.001	0.002	−0.012	−0.002
PEN-D	0.048	0.016	–	–	0.001	−0.013	−0.008	−0.004	−0.023	0.001	0.007
PEN-E	0.007	0.011	–	0.009	–	0.012	0.002	0.008	−0.003	0.025	0.012
PEN-F	0.040	−0.011	–	0.005	−0.018	–	0.010	0.003	−0.017	0.001	−0.012
PEN-G	0.041	**0.083**	–	−0.013	0.010	0.023	–	−0.016	0.000	0.031	0.023
PEN-H	–	–	–	–	–	–	–	–	−0.011	0.004	0.004
PEN-I	0.026	0.007	–	0.006	−0.022	−0.013	0.006	–	–	0.004	−0.020
PEN-J	−0.020	0.021	–	0.016	−0.004	0.011	0.015	–	0.002	–	−0.004
PEN-K	**0.062**	0.006	–	0.018	0.008	−0.017	0.026	–	0.007	0.036	–

Patches	ELS-A	ELS-B	ELS-C
(b) El Salobral locality			
ELS-A	–	0.014	−0.013
ELS-B	**0.057**	–	0.023
ELS-C	**0.034**	**0.095**	–

Patches PEN-H and PEN-C were not analyzed for females due to very low sample sizes ($n = 3$ in both cases). Values in bold are statistically significant after sequential Bonferroni correction ($\alpha = 0.05$)

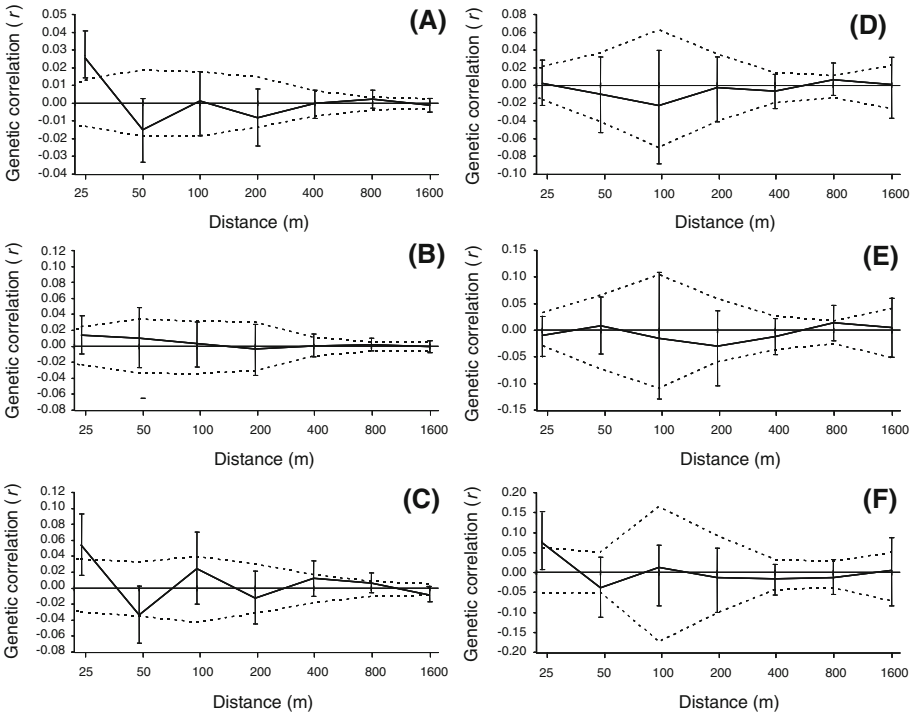

Fig. 2 Correlogram plots of the genetic correlation coefficient (r) as a function of distance for both sexes combined (**a**, **d**), males (**b**, **e**), and females (**c**, **f**) in each study locality (Peña Hueca: **a–c**; El Salobral: **d–f**). The permuted 95% confidence interval (*dashed lines*) and the bootstrapped 95% confidence *error bars* are also shown

males than in females. When data from both males and females were analyzed together, we neither found any significant pairwise F_{ST} after applying sequential Bonferroni correction (all $P > 0.05$). The overall F_{ST} was significant in Peña Hueca population ($F_{ST} = 0.031$; $P = 0.019$) but not in El Salobral ($F_{ST} = 0.019$; $P = 0.510$). When both sexes were analyzed separately, we found a significant overall F_{ST} for females in both populations (Peña Hueca: $F_{ST} = 0.076$; $P = 0.010$; El Salobral: $F_{ST} = 0.068$; $P = 0.048$), but not for males (Peña Hueca: $F_{ST} = 0.051$; $P = 0.171$; El Salobral: $F_{ST} = 0.030$; $P = 0.637$). Accordingly, mean patch pair-wise F_{ST} was significantly smaller in males than in females (Mann-Whitney U-test: Peña Hueca: $Z = -2.25$, $P = 0.024$; combining data from the two studied populations: $Z = -2.71$, $P = 0.006$). Mean F_{ST} values were also significantly lower for males than for females in most sampling patches (binomial test; Peña Hueca: $P = 0.019$; $n = 9$; combining data from the two studied populations: $P = 0.006$; $n = 11$). The randomization method implemented in FSTAT also revealed lower F_{ST} values in males than in females, although this difference between sexes was statistically significant for El Salobral population ($P = 0.037$) but only approached significance in Peña Hueca population ($P = 0.086$). Autocorrelation analyses found significantly positive r-values within the 25 m distance class when both sexes were combined into the analyses in Peña Hueca ($P = 0.003$; Fig. 2a) but not in El Salobral population ($P = 0.371$; Fig. 2d). Analyses using only male or female genotypes revealed a contrasting pattern between sexes: in both populations there was a significant kinship structure within the 25 m

distance class for females (Peña Hueca: $P = 0.002$, Fig. 2c; El Salobral: $P = 0.017$, Fig. 2f) but not for males (Peña Hueca: $P = 0.122$, Fig. 2b; El Salobral: $P = 0.721$, Fig. 2e).

Isolation by distance

Comparisons of pairwise genetic (F_{ST}) and Euclidean geographical distances between sampling patches in Peña Hueca locality revealed a significant isolation-by-distance pattern of genetic structure ($Z = 5.29$, $r = 0.306$, one-sided $P = 0.035$ from 10 000 randomizations) and a value of $r^2 = 0.093$ for RMA regression analysis. When we re-analyzed the data considering males and females separately we found a marginally significant positive correlation between genetic and geographic distances for females ($Z = 31.76$, $r = 0.306$, one-sided $P = 0.052$ from 10 000 randomizations; RMA $r^2 = 0.094$) but not for males ($Z = 6.86$, $r = 0.084$, one-sided $P = 0.309$ from 10 000 randomizations; RMA $r^2 = 0.007$) (Fig. 3). The slope values (b) of the relationship between pairwise genetic (F_{ST}) and Euclidean geographical distances were significantly smaller for males than for females (Mann-Whitney U-test: $Z = -1.99$, $P = 0.047$). The slope values were also significantly smaller for males than for females in eight out of the nine analyzed sampling patches (binomial test; $P = 0.019$; $n = 9$).

When we considered individuals rather than sampling patches, we found that the slope values of individual regressions of pairwise relatedness on geographical distance were significantly different from zero when both sexes were combined in Peña Hueca population (t-test; $t = -3.69$, $P < 0.001$; Figs. 4, 5) but not in El Salobral (t-test; $t = -0.27$, $P = 0.785$; Fig. 4A and 5A). When we re-analyzed the data considering male and female

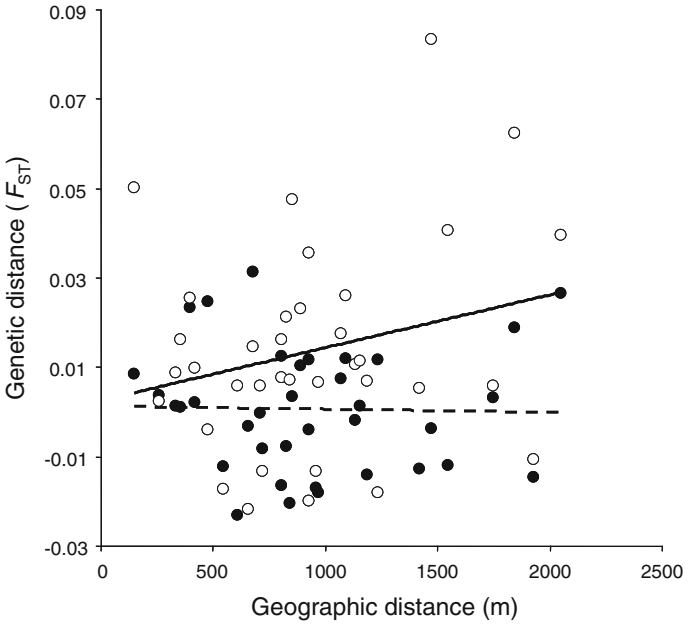

Fig. 3 Correlation between genetic (F_{ST}) and geographic distance for male (*solid circles*, *dashed regression line*) and female (*open circles*, *solid regression line*) individuals in Peña Hueca population. Reduced Major Axis (*RMA*) regression lines are showed

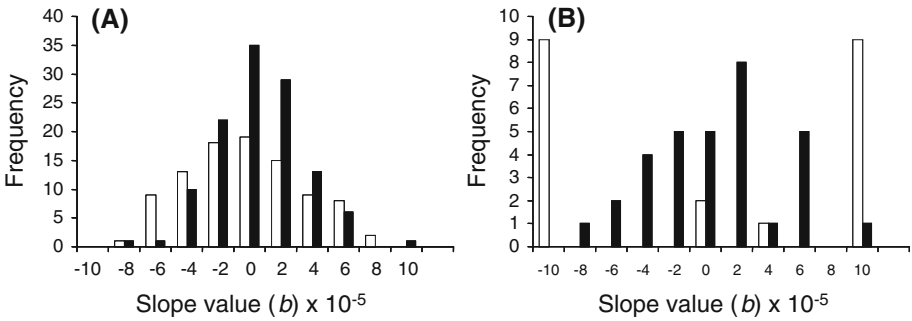

Fig. 4 Frequency distribution of the regression slope values (*b*) of relatedness on distance for male (*black bars*) and female (*white bars*) *M. wagneri* in **a** Peña Hueca and **b** El Salobral populations

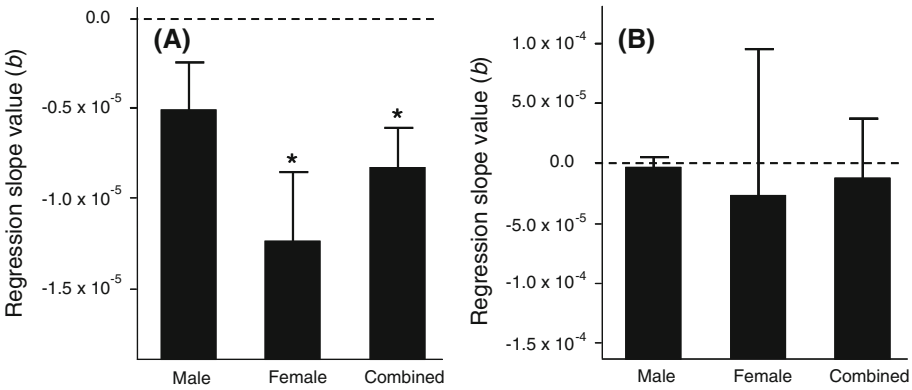

Fig. 5 Regression slope values (*b*) obtained from linear regression analyses between relatedness and Euclidean geographic distance for individual males, females, and both sexes combined in **a** Peña Hueca and **b** El Salobral populations. *Asterisks* mark observations that significantly departed from zero ($P < 0.05$)

genotypes separately we found that in Peña Hueca population the slopes values were significantly different from zero in females (*t*-test; $t = -3.23$, $P = 0.002$) but only marginally different in males (*t*-test; $t = -1.93$, $P = 0.056$) (Fig. 5A). However, in El Salobral population the slopes values were not significantly different from zero in males (*t*-test; $t = -0.45$, $P = 0.65$) or females (*t*-test; $t = -0.23$, $P = 0.822$). After controlling for patch identity (GLM: $F_{10,\ 200} = 2.19$; $P = 0.033$), the slope values of individual regressions differed between males and females in Peña Hueca population (GLM: $F_{1,\ 200} = 4.86$; $P = 0.030$). However, we found no significant difference in the slope values among patches (GLM: $F_{2,\ 48} = 2.24$; $P = 0.117$) or between males and females (GLM: $F_{1,\ 48} = 0.14$; $P = 0.707$) in El Salobral population.

Discussion

In this study we have found significant F_{ST} values within two populations of *M. wagneri*, suggesting restricted gene flow and dispersal within a local spatial scale. Patterns of isolation-by-distance at both patch scale and individual level within the larger and

intensively sampled population at Peña Hueca locality have also shown that genetic differentiation and genetic distance between individuals increase with geographical distance. Thus, information on fine spatial genetic structure in this population indicates subtle genetic differentiation which increases gradually with geographical distance, suggesting a local equilibrium between gene flow and drift (i.e. an isolation-by-distance pattern; Rousset 1997; Hutchison and Templeton 1999). However, isolation-by-distance analyses at the individual level were not significant in El Salobral population. This could be explained by the fact that this population extends over a smaller geographical area than Peña Hueca population (0.8 km in El Salobral *vs* 2.5 km in Peña Hueca; Fig. 1), which may have limited the number of pair-wise comparisons involving geographically distant individuals with comparatively lower genetic distance values. This, together with a small sample size, may have contributed to reduce the strength of the correlation between genetic and geographical distances in El Salobral population. El Salobral population also presents a higher distance between patches covered with inadequate habitats (i.e. cultures; Fig. 1), which may reduce inter-patch dispersal and increase the relative influence of genetic drift over gene flow (see Fig. 1-III in Hutchison and Templeton 1999). Thus, the spatial genetic structure within Peña Hueca population seems to follow an 'isolation-by-distance' pattern, whereas El Salobral population mostly resembles an 'island' model of spatial genetic structure (Hutchison and Templeton 1999; e.g. Van de Casteele and Matthysen 2006).

Knowledge on the fine-scale spatial genetic structure within populations has important applied implications, enabling the establishment of proper spatial scales for management and future conservation studies (Manel et al. 2003). *M. wagneri* presents an extremely fragmented distribution, forming small size and isolated populations which often show genetic signatures of demographic bottlenecks (Ortego et al. 2010). The observed pattern of genetic structure suggests a non-random distribution of genotypes at local spatial scales which can probably result in a smaller effective population size than census population numbers (Hedrick 1996; Harrison and Hastings 1996). This would be also particularly relevant for the study species in which stochastic or human induced habitat alterations (e.g. intense rainfall events, livestock grazing, etc.) often favour extinction/re-colonization dynamics at a local spatial scale (P. J. Cordero, unpublished data). These population dynamics, favoured by the small-sized patches of the relict environments where this species distributes (Ortego et al. 2009, 2010), together with the observed pattern of within population genetic substructure would result in more inbreeding than expected in a panmictic population with random mating and unrestricted dispersal (Wright 1969; Wright 1978; Coltman et al. 2003). This can further contribute to erode the genetic variability of the inherently small size populations of *M. wagneri* (Gilpin 1991; Hedrick 1996). The restricted dispersal distances revealed by general analyses on spatial genetic structure (Fig. 2) offers a proximate cause to explain the strong genetic structure observed in this species at larger geographical scales (Ortego et al. 2009, 2010). This also has important conservation implications: restricted dispersal capacity can limit the ability of this orthoptera to colonize new areas which can ultimately reduce the long-term persistence of their often highly isolated populations. Thus, the potential extinction of isolated populations will be hardly compensated by the rescuing effect of immigration, making this species and probably other co-distributed organisms with similar habitat requirements highly sensitive to stochastic phenomena (Ortego et al. 2010).

Genetic analyses have revealed a fine-scale spatial genetic substructure in females but not in males, suggesting that males are highly dispersive whereas females are mostly philopatric and remain in close spatial proximity to natal areas. Thus, as opposite to predictions, reversed sexual size dimorphism did not result in higher female dispersal in

this grasshopper species (Palo et al. 2004). Such a similar male-biased dispersal pattern has been previously found in the Mormon cricket (*Anabrus simplex*) (Bailey et al. 2007; but see Lorch et al. 2005; Sword et al. 2008). A possibility to explain this pattern is that female-biased predation has resulted in the observed male-biased dispersal even if both sexes tend to disperse equally (Palo et al. 2004) or that the smaller male size favours wind mediated passive male-dispersal (Robbins and Small 1981; Havel and Shurin 2004; Vanschoenwinkel et al. 2009). Other possibility is that the inferred male-biased dispersal pattern has evolved due to inter-male competition for mates (Greenwood 1980). This may occur because most studied populations show a male to female ratio much higher than parity and male nymphs become adults earlier than females (P. J. Cordero, unpublished data). Dispersal would increase mating probability and favour the access to a large number of females even at the expense of an increased risk of death (Greenwood 1980). Thus, males would ensure mating by increasing dispersal rates or distances and through active searching for available females. By contrast, females may benefit more than males from a familiarity with its birth site (Greenwood 1980). Females would gain advantage defending and searching optimal oviposition microhabitats in local areas where they have successfully survived during the egg stage, i.e. self-referential habitat choice. In orthoptera, female oviposition behaviour determines both the biotic and abiotic environment for egg development which, ultimately, is likely to have important consequences on offspring survival. Thus, females could gain significant advantage by occupying high quality familiar microhabitats to lay the clutches and this may explain their natal site-tenaciousness (Palo et al. 2004; Bailey et al. 2007). By contrast, males are not likely to defend any limiting resource as food is locally abundant in most studied populations and we have found no evidence of strong territorial behaviour (P. J. Cordero, unpublished data).

Inbreeding avoidance also offers a non-exclusive possible explanation for the observed sex-biased dispersal (Greenwood 1980). Dispersal could have evolved as a mechanism to reduce the chance of mating with genetically related individuals and minimize the detrimental consequences of such crosses (Greenwood 1980; Moore and Ali 1984; Pusey 1987; Lambin et al. 2001; Ortego et al. 2008b). Accordingly, several studies have found correlations between inbreeding or individual genetic diversity and different components of fitness (Charlesworth and Charlesworth 1987; Falconer and Mackay 1996; Chapman et al. 2009). However, it is difficult to predict the direction of the sex-bias if dispersal is maintained by the negative effects of inbreeding (Greenwood 1980). Directional predictions would be particularly difficult for species with short generation times (like *M. wagneri*) in which the cost of mating with genetically related individuals are not likely to differ between sexes (Greenwood 1980). Future studies on the association between individual genetic diversity and different components of fitness (parasitism, fecundity, body size) would help to resolve the potential negative consequences of mating with genetically related individuals in this species.

Overall, we have found a fine-scale spatial genetic substructure within the studied populations of *M. wagneri* which is likely to be mostly driven by female philopatry. Data on the breeding system and behaviour of *M. wagneri* (paternity skews, polygamy, cryptic mate choice, etc.) and more studies on other grasshopper species would help to explain the inferred sex-biased dispersal and establish the general prevalence of this pattern among orthoptera. This study provides evidence of restricted dispersal at a local spatial scale in a highly specialist orthoptera, highlighting the potential of molecular approaches to study the ecology and population structure in organisms for which a detailed monitoring of individuals is complicated.

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