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Fine-scale population structure, inbreeding risk and avoidance in a wild insect population

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Abstract

The ecological and evolutionary importance of fine-scale genetic structure within populations is increasingly appreciated. However, available data are largely restricted to wild vertebrates and eusocial insects. In addition, there is the expectation that most insects tend to have such large- and high-density populations and are so mobile that they are unlikely to face inbreeding risks through fine-scale population structuring. This has made the growing body of evidence for inbreeding avoidance in insects and its implication in mating systems evolution somewhat enigmatic. We present a 4-year study of a natural population of field crickets. Using detailed video monitoring combined with genotyping, we track the movement of all adults within the population and investigate genetic structure at a fine scale. We find some evidence for relatives being found in closer proximity, both across generations and within a single breeding season. Whilst incestuous matings are not avoided, population inbreeding is low, suggesting that mating is close to random and the limited fine-scale structure does not create significant inbreeding risk. Hence, there is little evidence for selective pressures associated with the evolution of inbreeding avoidance mechanisms in a closely related species.

Keywords: cricket, *Gryllus campestris*, inbreeding avoidance, microsatellite, population structure, video surveillance

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Introduction

Classical population models assume organisms live in discrete subpopulations within which individuals mate randomly (Wright 1978). However, the importance of structuring created by social interactions within subpopulations is increasingly appreciated (Sugg *et al.* 1996), and the resulting fine-scale genetic structure has been measured (Coltman *et al.* 2003; Peakall *et al.* 2003; Vekemans & Hardy 2004; Trontti *et al.* 2005; Hardy *et al.* 2008; Busch *et al.* 2009; Lee *et al.* 2009). Whilst it is easy to envisage fine-scale genetic structure in sessile organisms or in animals with complex societies (some mammals, birds, eusocial insects), the occurrence of such fine-scale structure in highly mobile, gregarious animals such as most insects is perhaps less intuitive (Hanski & Gaggiotti 2004). Here, we investigate whether such genetic structure exists in both space and time in a wild population of crickets.

Dispersal poses risks, such as straying into unsuitable habitat and away from potential mates, increased predation (Yoder *et al.* 2004) and decreased condition (Baker & Rao 2004); hence, even potentially highly mobile animals may tend to move less than their locomotory capacities allow. If animals do not disperse, this can lead to fine-scale genetic structure, the consequences of which are both ecological and evolutionary. For example, genetic structuring can influence effective population size and rate of loss of genetic variation (Chesser *et al.* 1993; Sugg *et al.* 1996). The extent of local adaptation

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may be highly dependent on the amount of fine-scale genetic structuring (Hendry *et al.* 2001). Increased interactions with kin may be positive (e.g. cooperation) or negative (e.g. competition) (Lambin *et al.* 2001) and may increase potential for, or be a product of, kin selection (Morin *et al.* 1994; Högland & Shorey 2003). Indeed, constraints on dispersal have been implicated in the evolution of sociality (Perrin & Lehmann 2001).

Perhaps the most critical consequence of genetic structuring, in both the short and long term, is increased inbreeding risk. Inbreeding can have deleterious effects on individual fitness (Roff 1997) and may even play a role in population extinction (Frankham 2005; Wright *et al.* 2008), making understanding the prevalence of inbreeding risks in natural systems an issue of importance for conservation (Hedrick & Kalinowski 2000). For example, in the Finnish metapopulation of Glanville fritillary (*Melitaea cinxia*) butterflies, decreasing heterozygosity was associated with the extinction of individual population patches (Saccheri *et al.* 1998).

Dispersal by one or both sexes, and therefore the breakdown of fine-scale genetic structure, is seen as one of a number of strategies to avoid inbreeding (Pusey & Wolf 1996). Hence, if fine-scale genetic structure is observed, it will not necessarily lead to inbreeding, or subsequently to inbreeding depression, but might create selection for inbreeding avoidance mechanisms that do not require individuals to avoid coming into contact, such as rejecting sperm from relatives (Pizzari *et al.* 2004; Bretman *et al.* 2009) or selectively aborting inbred offspring (Zeh & Zeh 2006). Insects have evolved kin recognition systems, often involving cuticular hydrocarbons (Howard & Blomquist 2005), and these systems are not only confined to eusoical insects (e.g. Pfennig & Reeve 1989; Lihoreau & Rivault 2009). This implies that kin interactions are important, although inbreeding avoidance is only one potential selective force. Among insects, inbreeding avoidance has been studied most in field crickets where females have been found to prefer the scent of unrelated males (Simmons 1989, 1991) and also preferentially store sperm from unrelated males (Bretman *et al.* 2009). Hence, there is evidence for the evolution of two inbreeding avoidance mechanisms in field crickets, raising the question of how acute inbreeding risks are in the wild and whether such avoidance can be observed there.

We have been studying a small (50–180 individuals per year) population of a univoltine field cricket (*Gryllus campestris*, L.) that lives in and around burrows in a lowland meadow where it has been continuously present for at least 40 years. This cricket is declining and red-listed through much of Northern and Central Europe (Witzenberger & Hochkirch 2008). Work at our field site in Northern Spain combines 4 years of detailed observational data from a network of up to 96 infrared video cameras with a comprehensive pedigree based on microsatellite markers (Rodríguez-Muñoz *et al.* 2010). Adults emerge during April and May and have a mating season of about 10 weeks (the maximum adult lifespan we have observed is 76 days). Eggs hatch by early summer and nymphs grow until October–November, when they re-treat to within their burrows and overwinter in diapause. Growth resumes at the end of the winter, and nymphs go through 1–2 final instars until adult eclosion. Although this species is mostly flightless, they are fairly robust (2–3 cm long), can run rapidly and have been observed to colonize sites more than 1 km from a source population (Witzenberger & Hochkirch 2008). However, we expect migration rates in our study population to be low as it is surrounded by unsuitable habitat, and our observed immigration rates of adults are very low (Rodríguez-Muñoz *et al.* 2010). Using this population, we have so far confirmed the fundamental prediction that males vary more in their reproductive success than females [for the 2006 parental cohort, the mean (±standard error) number of adult offspring for males was 1.92 (0.43) and for females, 1.79 (0.29)] and that both sexes benefit from mating with multiple partners. We have also found that the factors that predict male success in gaining mates differ from those that predict the number of offspring males leave (Rodríguez-Muñoz *et al.* 2010).

Using detailed observational data combined with genotyping, we track the movement of all adults within the population and determine genetic structure at a much finer scale than has previously been attempted, over distances of a few metres, both between multiple discrete generations and within a generation across the breeding season. Fine-scale analysis of relatedness has previously been carried out in a butterfly metapopulation where patches can be founded by as few as one individual and hence can consist only of siblings (Hanski *et al.* 1995), but have not been attempted in long-term stable insect populations. Laboratory studies of a closely related species (*G. bimaculatus*) have revealed a role for the post-copulatory inbreeding avoidance in field crickets through differential sperm storage (Tregenza & Wedell 2002; Bretman *et al.* 2004, 2009) but whether a mobile animal such as this, with no parental care, faces an inbreeding risk in the wild has been a critical gap in our knowledge. This study, therefore, forms the first step towards integrating these laboratory studies with field biology.

Materials and methods

Study site

Our field site is a meadow located in Asturias (Northern Spain) and is isolated from similar habitat by a railway,

a road and an orchard. The cricket population occupies an approximately flat area of around 800 m^2 (40 × 20 m) surrounded by trees and a north facing slope where crickets are very rarely found. Although there are other suitable habitats on neighbouring properties, migration seems to be limited by the existence of a shady, unsuitable area around most of the meadow's perimeter, with the only open side bordering the entrance road. We have only found three crickets outside the main area during the breeding season in 4 years of careful searching, and the largest number of possible immigrants we have recorded (identified by their lack of an ID tag) in a single year is 6, suggesting that immigration is also very limited (Rodríguez-Muñoz *et al.* 2010).

For 4 years from 2006 to 2009, we have monitored the entire adult season, catching newly enclosed adults, individually marking them and taking a very small (2 mm) clipping from the hind leg for genetic analysis. Soon after eclosion, all crickets are captured and tagged by gently abrading their thorax integument with very fine-grade sandpaper before applying the cyanoacrylate adhesive. We extensively trialed this method in the laboratory before employing it in the field, and we are confident in its efficacy as each year we have seen a maximum of six untagged adults. Up to 96 infrared video cameras are deployed in the field recording cricket activity at burrows (see Rodríguez-Muñoz *et al.* 2010). These videos allow us to observe all adult behaviours that occur at burrows, such as fighting, mating, egg laying and predation; thus, we repeatedly recapture tagged crickets until they are observed to be predated or entirely disappear. We have mapped adult locations and so can derive spatial distances between crickets in the population. Crickets frequently move among burrows several times a week, and our video surveillance allows us to record these movements. Mapping was carried out by measuring the distance from every burrow to each of two or three reference posts with known coordinates using a laser measuring device. Burrow coordinates were then calculated via trigonometry, deriving three complimentary estimates of position and checked against approximate maps made by recording the positions of burrows relative to one another by eye (Data S1, Supporting information).

Microsatellite analysis

We extracted DNA using a salting out protocol (Strassmann *et al.* 1999) and eluted the pellet in 20 μL autoclaved, double-deionized (dd) H_2O. As the sample was necessarily small, we did not quantify DNA, but amplified as a one in five dilution from the stock. Individuals were genotyped at 14 loci [*Gbim4* and *15* (Dawson *et al.* 2003); *Gbim21*, *26*, *29*, *33*, *49*, *52*, *53*, *57*, *59*, *66*, *71* and *72* (Bretman *et al.* 2008)]. To ensure, we obtained the highest-quality data possible, and each individual was genotyped in three separate reactions per locus. Loci were amplified in seven sets (Table S1, Supporting information). Each 10 μL PCR contained 0.2 mM of each dNTP, 1.0 mM $MgCl_2$ and 0.25 units of *Taq* DNA polymerase (Yorkshire Biosciences) in the manufacturer's buffer (final concentrations 100 mM Tris–Cl, 500 mM KCl, 1% Triton X-100). Primers were added in differing concentrations (Table 1). PCRs were performed on a PX2 Thermo cycler (Thermo Electron). To each 10 μL reaction, we added 10 μL of ddH_20 and then multiplexed them into three sets (Table S1, Supporting information) by adding each constituent PCR product in equal volume. Products were then run on an ABI 3130XL genetic sequencer (Applied Biosystems) and analysed using GeneMapper v 4.0 software (Applied Biosystems).

The three genotypes for each locus were collated, and a consensus genotype was formed for each individual. The general rule for the consensus genotype was that the majority genotype was taken (i.e. the genotype of 2/3 reactions). For heterozygotes, both alleles had to have been amplified in another reaction (although this could be a heterozygote and two different homozygotes); homozygotes had to only ever have been typed as homozygotes. This produced successful genotypes for 95% of parental genotypes (149 individuals, genotyped three times each at 14 loci) and 98% of offspring genotypes (184 individuals, genotyped three times each at 14 loci). Allele frequencies and tests for null alleles were obtained using Cervus v 3.0 (Kalinowski *et al.* 2007). we confirmed these were true nulls through parent–offspring mismatches in the pedigree. If these loci were not nulls, but the excess of homozygotes was instead indicative of inbreeding, then these loci would not show a large number of parent–offspring mismatches where parent and offspring are 'homozygous' (in

Table 1 Correlations between spatial distance and relatedness (Wang's *r*)

Year	Spearman's rho	*N*	*P*
2006	−0.045 (−0.042)	4896	0.002 (0.003)
2007	−0.026 (−0.034)	5897	0.042 (0.009)
2008	−0.067	594	0.101
2009	−0.082	5712	<0.0001

Spatial distances are derived from the mean sighted position in 2006–2008, with the last position given in parentheses for 2006 and 2007; for 2009, the distance is derived from the first sighted position.

reality, hemizygous) for different alleles. Loci *Gbim59* and *71* were found to be sex linked (crickets have an XO sex determination, so all males were homozygotes); hence, inferred relationships and estimates of pairwise relatedness for pairs other than mother–daughter would be incorrect using these loci. Therefore, both loci were removed from further analysis. *Gbim21* had a high incidence of null alleles and was also removed from further analysis.

Relatedness assignment

For adults found in the years 2007–2009, paternity and maternity were inferred from genetic similarities at 11 microsatellite loci using the software COLONY2 (Wang 2004; Wang & Santure 2009). This programme maximizes group likelihoods and returns estimates of paternal, maternal, full and half-sibling relationships [for a detailed description, see (Wang & Santure 2009)]. COLONY2 accounts for genotyping errors attributed to null alleles and other stochastic errors separately on a locus-specific basis. Error rates were estimated using the three replicate genotypes. Stochastic error rates were categorized as the proportion of mistyped alleles (those appearing with two different scores). We identified loci with nulls as discussed earlier. It was noted that these occurred mainly in 3 loci (*Gbim26*, *57* and *66*, Table S2, Supporting information); hence, the null rates were set separately for these loci to 12% and 1% for the rest of the loci. From field observations, the size of the unsampled population was predicted to be small—over the last 4 years, the maximum number of crickets observed during the breeding season that were not seen undergoing their final moult in the meadow (and hence identified as possible immigrants) in any given year has been 8. As such, we conservatively specified the probability of the actual father and mother being genotyped as 0.75. Paternity and maternity assignments were only accepted if the confidence in the individual assignments was ≥80%, and the majority were more than 99% (mean ± SE of assignments accepted 0.994 ± 0.001).

Throughout, we use the word 'dyad' to refer to all male–female pairwise spatial and genetic comparisons. Using the derived pedigrees, we assigned cricket dyads to be either 'related' (either full or half siblings) or 'unrelated'. As 2006 was the first year of study, no pedigree could be determined. To assign relatedness for this year, we calculated pairwise relatedness coefficients (Wang's *r*) using MER v. 3 (Wang 2002). As a conservative definition, we considered individuals to be related where $r \geq 0.25$, the expected mean value for half sibs. This meant we categorized relatives such as cousins as unrelated and resulted in a similar proportion of related dyads as in 2007 (0.07 in 2006 and 0.06 in 2007).

Statistical analysis

Statistical analysis was performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and the MSEXCEL plug-in POPTOOLS (http://www.poptools.org/). As we are concerned principally with inbreeding risk, we present data for male–female dyads only. To test for genetic structure, as there was necessarily a large difference in sample size for related and unrelated groups (in the region of an order of magnitude) we used randomisation tests (Manly 1997). ANOVA was performed on real spatial distances between related and unrelated dyads, the data resampled without replacement (shuffled between rows and columns) and ANOVA performed on the resampled data. This was repeated 10^5 times, and the significance was calculated as the proportion of repetitions in which the *F* value associated with the resampled data equalled or exceeded that of the real data *F* value (significance values were doubled if <0.05 to allow for a two tailed test). In addition, we performed Spearman's rank correlations between spatial distances and relatedness values (Wang's *r*) for each dyad. We also used χ^2 tests to test differences in propensity to mate between related and unrelated male–female dyads and to investigate whether there was a difference in number of surviving offspring from related and unrelated parents. To assess whether the population was significantly inbred, we used only the eight loci without high null allele values. Wright's inbreeding coefficient [F_{IS} (Wright 1965)] was determined using the formula $[1-(H_o/H_e)]$, averaged across loci, and using Nei's sample size correction for expected heterozygosity. F_{IS} was tested for deviations from 0 using one-sample *t* tests, both for each year independently and for all years combined. Genetic neighbourhood size was calculated using the formula $2R$ (the diameter of the neighbourhood) $= 2\sqrt{4/3}*S^2*T$, where *S* is the variance of breeding displacement (distance travelled by a cricket over the adult season) is the adult lifetime period over which it can produce offspring (one season) (Lodé & Peltier 2005).

If the observed population structure created an inbreeding risk, simulating matings between the spatially closest observed dyads should produce an inbreeding coefficient >0 and at least as great as the observed inbreeding coefficient of the population. For these simulations [run in R version 2.11 (Ihaka & Gentleman 1996)], we paired each female with the male that she was closest to at her first observation. In this way, all females were represented once but males could be represented more than once or not at all, to reflect the greater variance in reproductive output by males (Rodríguez-Muñoz *et al.* 2010). Offspring were then simulated by randomly assigning an allele from each

parent at each locus, and this process was repeated 1000 times. For 2006 and 2007, this was repeated using the mean observed positions of the parents, as well as the first position they were observed at. F_{IS} was calculated and compared with the observed levels of inbreeding.

We examined how far nymphs dispersed relative to the expectation if they had occupied a burrow at random. Simulated nymphal dispersal was modelled by retaining the observed position of the mother and assigning nymphs to a random available burrow, without replacement to reflect that burrows were not multiply occupied. An observation that real nymph dispersal distances were as great as distances resulting from random occupation of burrows (rather than tending to be local to their mothers burrow as one might expect) would be consistent with the idea that nymphal dispersal was a mechanism to avoid close inbreeding. For females that produced offspring, a relative measure of female home range size was calculated. Firstly, we found the centroid that minimized the total distance between the female's position and that point, so if a female spent an hour at one burrow and 10 h at another burrow the point would be 10× nearer the 2nd burrow. Secondly, we took the distance of the female from the centroid averaged over her adult life, and we use this distance to describe her home range. This creates a measure in which the female is typically within the radius described [females were observed within this range 81% of the time (±1.5%)] and which reflects her typical position rather than being dominated by occasional forays to more distant areas.

Results

Summary information for all loci can be found in Table S2 (Supporting information).

Cricket movement

Comparing the adult emergence site of offspring with the mean position of their mother, in 2007, nymphs moved a median distance of 9.08 m (0.84–36.13 m range) and in 2008 10.51 m (2.48–18.78 m) (Fig. 1a), with no difference between the years (Mann–Whitney *U*-test $Z = 0.095$, $N = 164$, $P = 0.925$). There was no difference between sexes (2007 females 9.24 m, males 8.98 m, $Z = 0.782$, $N = 138$, $P = 0.434$; 2008 females 10.18 m, males 10.96 m, $t = 0.352$, d.f. = 18, $P = 0.729$). Simulated nymphal dispersal gave values greater than observed, (mean ± SE 2007 14.03 ± 0.02 m, compared to observed distance $P = 1$; 2008 11.91 ± 0.04 m compared to observed distance $P = 0.95$) indicating that nymphs dispersed slightly less than if they had occupied a burrow at random.

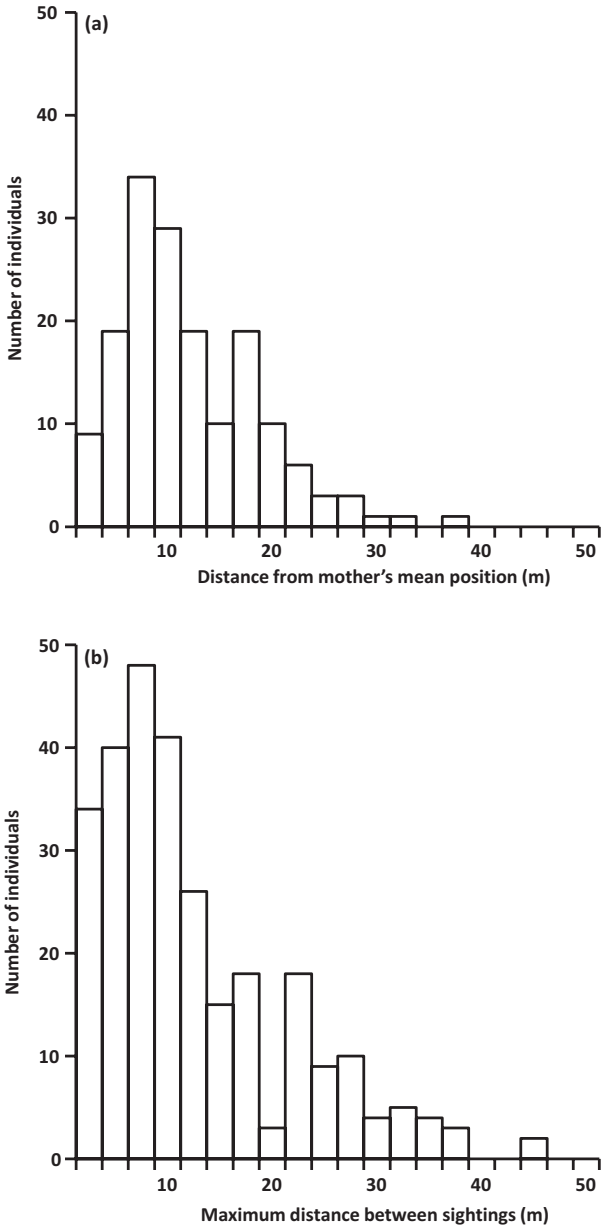

Fig. 1 Distances moved by individual crickets, measured for (a) nymphs as the distance from emergence site to mother's mean position and for (b) adults as the maximum distance between sightings. Data combine the years 2006–2008. Adults that were only seen at one burrow were removed from the analysis.

During the mating season, considering only adults that were seen at more than one burrow, the median (and range) of maximum distances between sightings was 9.2 m (0.2 –35.9 m) in 2006, 8.1 m (0.2–44.9 m) in 2007 and 9.0 m (0.7–43.7 m) in 2008, with no significant difference between years (ANOVA on square root transformed

data $F_{2,277} = 0.67$, $P = 0.511$: Fig. 1b). We calculated the area in which females may have laid eggs as 44.6 m^2 (±10.37) in 2006, 69.5 m^2 (±44.11) in 2007 and 71.59 m^2 (±24.86) in 2008. The radius of the genetic neighbourhood (R) was 10.50 m in 2006, 10.54 m in 2007 and 9.45 m in 2008, representing approximately half the total field area.

Spatial distance and relatedness

In three of the 4 years of study, related male–female dyads (sharing at least one parent in 2007–2009, or $r > 0.25$ in 2006) were found significantly closer together than unrelated dyads within their ≈40 × 20 m meadow by a mean of ≈1 m (in the context of the mean distance between individuals being 12–14 m, Fig. 2 randomisation tests; 2006, dependent F value ≥ test F value in $1337/10^5$ iterations $P = 0.027$; 2007, $378/10^5$ iterations $P = 0.0076$; 2009, $2/10^5$ iterations $P = 0.00004$; Fig. 2). In 2008, there was no difference in distance between related and unrelated dyads (dependent F value ≥ test F value $61632/10^5$ iterations $P = 0.616$, Fig. 2); however, 2008 was unusual in that the population size was greatly reduced (51 individuals compared with 151 in 2006, 188 in 2007 and 152 in 2009). In 2006, 2007 and 2008, data were available for the position of the last observation for each cricket, and the pattern of spatial structure (or lack of it) persisted throughout the season (distance of dyads at the last observed position: 2006, dependent F value ≥ test F value $359/10^5$ iterations $P = 0.007$; 2007, $34/10^5$ iterations $P = 0.0007$: 2008, $43140/10^5$ iterations $P = 0.431$). These findings were supported by correlations between spatial distances and Wang's r values (Table 1), with weak but significant negative correlations in 2006, 2007 and 2009, but no significant correlation in 2008.

Mating propensity

Data on dyads being seen together and mating were available for 2006 and 2007. In 2006, dyads seen together were more related than those not seen together

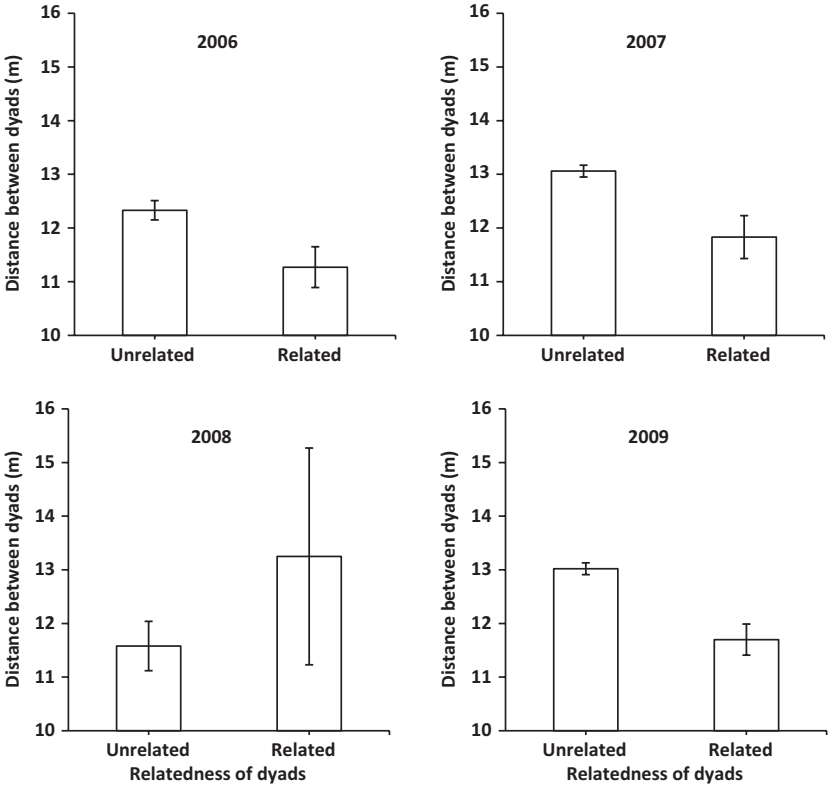

Fig. 2 Spatial distance and relatedness between dyads of crickets over 4 years; 2006–2009. Spatial distance (m, mean ± 1 standard error) was measured for the mean (2006–2008) or first (2009) location in which the cricket was observed. Relatedness for 2007–2009 is derived from a pedigree using the programme COLONY2, related individuals are at least half siblings. Only dyads where either both mothers or both fathers were assigned were included. In 2006, no pedigree could be determined; hence, related individuals are those with a pairwise relatedness value (Wang's r) of at least 0.25. In 2006, 2007 and 2009, the distance between related dyads is significantly smaller than unrelated dyads.

(Mann–Whitney *U*-test $Z = 3.451$, $N = 4896$, $P = 0.001$) but the mean relatedness (Wang's *r*) of those seen together was not significantly >0 (Wilcoxon signed ranks test, $V = 3732$ $N = 112$, P = 0.840).

Of those male–female pairs observed together, related pairs ($r > 0.25$) were more likely to mate (for related pairs 10/13 mated compared with 83/190 of unrelated pairs, $\chi^2 = 5.42$, d.f. = 1, $P = 0.04$). In 2007, there was no difference in relatedness between dyads seen or not seen together (Mann–Whitney *U*-test $Z = 0.123$, $N = 5897$, $P = 0.902$), and the mean relatedness of dyads seen together was significantly <0 (Wilcoxon signed ranks test, $V = 22187$, $N = 259$, $P = 0.028$). Likewise, there was no difference in propensity to mate between related (sharing at least one parent) and unrelated pairs (9/17 of related pairs mated compared with 159/253 of unrelated pairs, $\chi^2 = 0.66$, d.f. = 1, $P = 0.41$).

Inbreeding

Treating years separately, there was no evidence in any year of significant population inbreeding, as the inbreeding coefficient (F_{IS}) was not significantly >0 (Table 2). Likewise, pooling the data across years showed no evidence of significant inbreeding (Table 2). When we simulated offspring from the closest dyads, the simulated F_{IS} was slightly positive, but never significantly different to the observed F_{IS}, either for the first sighted position or the mean sighting in 2006 and 2007 (Table 2).

There was no evidence that offspring from related parents suffered higher mortality than those from unrelated parents, although this is based on limited data. Combining data from 2006 and 2007, 4/72-related male–female pairings produced offspring compared with 62/10277-unrelated male–female pairings ($\chi^2 = 0.22$, d.f. = 1, $P = 0.64$). Although we applied Yates' correction for expected values lower than 5, we advocate caution in interpreting these results.

Table 2 Inbreeding coefficients (F_{IS}) for (a) observed data; (b) simulated data

(a)

		Comparison to $F_{IS} = 0$		
Year	F_{IS} mean (SE)	*t*	d.f.	*P*
2009	0.05 (0.03)	1.57	7	0.16
2007	−0.003 (0.03)	−0.11	7	0.91
2008	0.02 (0.04)	0.45	7	0.67
2009	0.04 (0.05)	0.78	7	0.46
All years pooled	0.03 (0.02)	1.87	7	0.10

(b)

Data source	Year	F_{IS} mean (SE)	Comparison with observed data *P*
First position	2006	0.004 (0.0008)	0.60
	2007	0.02 (0.0009)	0.51
	2008	0.02 ± 0.0014	0.34
	2009	0.03 (0.0008)	NA
Mean position	2007	0.004 (0.0008)	0.56
	2008	−0.01 (0.0007)	0.13

Mean F_{IS} was compared to 0 (no inbreeding) for observed data. Simulated data were obtained by simulating offspring from the closest observed dyads, using the first position or mean position sighted. Simulated data were compared with the observed data; hence, the F_{IS} from offspring simulated from parents in 2006 was compared with the observed values for 2007 using randomization tests (1000 iterations).

Discussion

By individually marking and observing individuals throughout their adult lifespan, we were able to investigate extremely fine-scale genetic structure in a wild population of a mobile insect. Related dyads were found closer together in three (2006, 2007, 2009) out of 4 years of study. Importantly, in 2006 and 2007 (where data were available), the pattern is observed using the mean and last sighted position; hence, although it might be expected that clutch mates will be more likely to be first sighted as adults in close proximity because they were together as eggs, the pattern is not simply a result of restricted nymphal movement. In addition, in 2006, male–female dyads seen together were more likely to mate if related than if unrelated (and those seen together also have a significantly greater relatedness, although this is not significantly >0) and showed no difference in propensity to mate with respect to relatedness in 2007; hence, there is no evidence of precopulatory inbreeding avoidance. Taken together, this suggests that these crickets face an inbreeding risk through random pairing in a small population, which they fail to avoid before mating. However, we found no evidence of significant inbreeding, either treating years separately or pooling data from all 4 years, even in the year following the population crash (2009, in which 13% of pairs were at least half sibs, double the number compared with the previous years). We think it unlikely that this lack of a signature of inbreeding is attributed to migration as our observed adult migration rates are low. Nevertheless, simulating cohorts derived from male to female dyads sighted closest together did not result in greater inbreeding than detected in the real population, so the structure we observe would not lead to a highly inbred population, at least over one generation.

The costs of inbreeding and the mechanism of inbreeding avoidance have been measured in a sister species to the one we observed in the wild, *G. bimaculatus*. Female *G. bimaculatus* are highly polyandrous in the laboratory and field (Bretman & Tregenza 2005) and will readily mate with their siblings, despite lower viability of inbred offspring (Tregenza & Wedell 2002). Polyandry allows females to overcome this cost, as females mated to a sibling and an unrelated male achieve the same hatching success as females mated to only unrelated males (Tregenza & Wedell 2002). We have recently shown that females bias sperm storage in favour of unrelated males (Bretman *et al.* 2009), leading to higher paternity success for unrelated males (Bretman *et al.* 2004, 2009). Such post-copulatory inbreeding avoidance has been suggested in a variety of species (e.g. Olsson *et al.* 1996; Stockley 1999; Kraaijeveld-Smit *et al.* 2002; Mack *et al.* 2002; Thuman & Griffith 2005; Simmons *et al.* 2006; Jehle *et al.* 2007); yet, the mechanisms remain relatively unexplored (Pizzari *et al.* 2004; Zeh & Zeh 2006; Bretman *et al.* 2009). We do not know whether the same mechanism of post-copulatory inbreeding avoidance is used by *G. campestris*; however, the two species are very closely related [all of the microsatellite loci used in this study were developed from a *G. bimaculatus* library, and the two species will hybridize in the laboratory and produce fertile offspring (von Hörmann-Heck 1957)]. An alternative explanation for the lack of inbreeding in this population is that inbred offspring may suffer increased mortality before adulthood, although we do not find any difference in the number of offspring from related parents observed in the next year relative to all possible parental dyads. In the laboratory, *G. bimaculatus* mating to a full sibling suffer decreased hatching success (Tregenza & Wedell 2002), and inbreeding is known to affect life history traits such as development time and longevity (Wright *et al.* 2008). As we could only census adults in the following year, if inbred offspring did die before becoming adult we would not sample them. An additional issue is that Gryllid crickets produce flighted and flightless morphs, and the proportion of these two morphs has been related to environmental conditions such as density (reviewed in Harrison 1980). A key difference between *G. campestris* and *G. bimaculatus* is that flighted polymorphisms are much rarer in *G. campestris* (we have never observed them) and both sexes are far more territorial. This might tend to increase structure in *G. campestris* relative to *G. bimaculatus*. Nevertheless, our simulations suggest that the spatial distribution in our population keeps inbreeding risk low, rendering the evolution of the post-mating inbreeding avoidance mechanism in *G. bimaculatus* even more remarkable.

In the last few decades, we have gained many insights into the spatial ecology of insects living in networks of populations characterized by extinction and recolonisation events, particularly through studies of the Finnish *Melitaea cinxia* metapopulation (Hanski 1999; Orsini *et al.* 2008). Our findings indicate that populations may have genetic structure even below the subpopulation level, but that movement of even this relatively sessile insect around the population is considerable. From our calculations of genetic neighbourhood size and mother–offspring or individual distances over the season, there is certainly the potential for complete mixing to occur within this population. Our assumption that this population is potentially panmictic (that is individuals could mate with any other individual in the field) is largely borne out. Previously described genetic structure on this spatial scale in insects has been confined to two studies of ants which found structure at <30 m [*Plagiolepsis pygmaeaI* (Trontti *et al.* 2005)] and <250 m [*Cataglyphis cursor* (Hardy *et al.* 2008)]. Ant species may be unusual relative to insects in general, as it has been suggested that inbreeding may be beneficial for eusocial insects to promote cooperation and altruism (Bourke & Franks 1995), and that even in species in which colonies have multiple queens, reduced female dispersal and adoption of daughter queens promote beneficial structure (Trontti *et al.* 2005). These benefits of structure will probably not be broadly applicable as inbreeding is generally detrimental (Roff 1997; Frankham 2005), and hence our crickets may be more likely to be representative of insects in general.

In 2008, even the limited genetic structure indicated by our comparison of related and unrelated dyads was absent and the population size was one-third that of other years and consequently the density was substantially lower. We need further years of data before we can do more than speculate about why the population declined so severely, but as numbers returned to previous levels the following year we suggest this was a short-term fluctuation, possibly caused by weather conditions in 2008. Such fluctuations are thought to be common in *G. campestris* and have been attributed to climatic conditions (Remmert 1992). This clearly demonstrates the need to integrate ecological and evolutionary studies, as advocated by Siepielski *et al.* (2009). This pattern is in contrast to that previously observed in plants (Williams 1994) and mammals (Nussey *et al.* 2005; Busch *et al.* 2009). Busch *et al.* (2009) found that over 14 years of study in two populations of banner-tailed kangaroo rat (*Dipodomys spectabilis*) structure broke down when population densities were high, as kin groups overlapped, and the authors predicted this would be the case for other animals. Nussey *et al.* (2005) found declining structure between subgroups of females red deer (*Cervus elaphus*) associated with an increase in population size and decreased polygyny. In

contrast, our results indicate a breakdown of structure when density was low. This was not caused by there being fewer related groups in this year (the proportion of related individuals was the same as in other years) or a decrease in adult dispersal compared with other years. It is possible that our population is unusual in that there are physical barriers that limit dispersal in and out of the meadow (see Materials and methods and Data S1, Supporting information). The evidence for fine-scale structure we observe may be a product of the crickets being unable to move as much as they would in open habitat, in which case we might expect larger meadows to have even lower inbreeding risks and limited selection for inbreeding avoidance mechanisms.

Metapopulation ecology is now an established part of conservation research (McCullough 1996); indeed, a recent attempt to model conservation priorities used three spatial scales, 10 × 10 km, regional and metapopulation level (Cabeza *et al.* 2010). Our findings suggest that at least for some conservation objectives, a further, within subpopulation, level should be considered. We find that although the fine-scale genetic structure we observe marginally increases inbreeding risk over a completely panmictic population, the population does not suffer from substantial realized inbreeding. A further lesson from our data is that although these animals can move considerable distances (Witzenberger & Hochkirch 2008), many individuals move only a few metres; hence, translocation may be necessary to establish new populations (Hochkirch *et al.* 2007).

We have shown that in a wild insect population, relatives do occasionally encounter one another and mate and produce offspring. However, we find only very weak population structure, and our analysis suggests that the proximity of related individuals does not lead to substantial inbreeding or risk of inbreeding. Hence, the evolution of post-copulatory inbreeding avoidance mechanisms in a closely related cricket with an ecology that is apparently less likely to create inbreeding risks remains enigmatic.

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Data accessibility

Spatial distances and microsatellite data: deposited at Dryad doi: 10.5061/dryad.9116.

Supporting information

Additional supporting information may be found in the online version of this article.

Data S1 Detailed methods, Fig. S1 field map, Fig. S2 individual movement range, Fig. S3 photo of tagged crickets.

Table S1 PCR multiplexes and primer concentrations.

Table S2 Summary of locus information for each year and all 4 years combined.

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