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Outbreaks, gene flow and effective population size in the migratory locust, *Locusta migratoria*: a regional-scale comparative survey

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Abstract

The potential effect of population outbreaks on within and between genetic variation of populations in pest species has rarely been assessed. In this study, we compare patterns of genetic variation in different sets of historically frequently outbreaking and rarely outbreaking populations of an agricultural pest of major importance, the migratory locust, *Locusta migratoria*. We analyse genetic variation within and between 24 populations at 14 microsatellites in Western Europe, where only ancient and low-intensity outbreaks have been reported (non-outbreaking populations), and in Madagascar and Northern China, where frequent and intense outbreak events have been recorded over the last century (outbreaking populations). Our comparative survey shows that (i) the long-term effective population size is similar in outbreaking and non-outbreaking populations, as evidenced by similar estimates of genetic diversity, and (ii) gene flow is substantially larger among outbreaking populations than among non-outbreaking populations, as evidenced by a fourfold to 30-fold difference in F_{ST} values. We discuss the implications for population dynamics and the consequences for management strategies of the observed patterns of genetic variation in *L. migratoria* populations with contrasting historical outbreak frequency and extent.

Keywords: gene flow, population structure, outbreak, pest, microsatellites, null alleles, locust

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Introduction

Species with fluctuating population size are of major concern to agriculture (e.g. some cyclic microtine rodents; pest moths; swarming locusts) and to human and animal health (e.g. epizootic parasites), particularly in developing countries. Thomas (1999) stressed the need for developing management strategies for both pests and diseases that adopt an ecological approach built around a fundamental understanding of the population biology of the concerned species. The design of preventive management strategies that would reduce environmental and monetary costs, in particular, requires a clear understanding of the spatial dynamics of threatening species (Hunter 2004). The study of population dynamics and structure of threatening species can be addressed using direct ecological approaches, such as density surveys or mark–release–recapture methods. The analysis of field density estimates is extremely useful to infer changes in population density and to correlate these to ecological factors (Dempster 1963), but cannot be applied to assess with precision patterns of movements. The implementation of mark–release–recapture often fails in fluctuating populations, due to their great dispersal ability (e.g. swarming locusts; Duranton *et al*. 1979) and/or to their low density during the remission period (e.g. cyclic microtine rodents; Wassenaar & Hobson 1998). In these situations, the use of population genetics approaches may represent a useful alternative.

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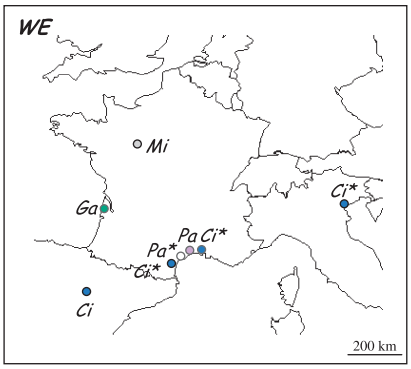
Simulation and analytical studies have shown that temporal heterogeneities in demographic parameters, such as effective population size and migration rate, may have large effects on genetic variation within and between populations (Whitlock 1992; Ehrich & Jorde 2005). The sensitivity to demographic fluctuations of statistics summarizing genetic variation depends on dispersal characteristics, effective population sizes, strength of demographic fluctuations, and properties of the genetic markers (Slatkin 1994; Rousset 2003; Leblois *et al*. 2004, 2006). Often, there is still a lack of clear theoretical expectations when organisms display demographic patterns that greatly depart from mutation–drift–migration equilibrium (Leblois *et al*. 2004, 2006). It may be difficult to interpret measurements of genetic variation in terms of biological parameters, such as migration rates or effective population sizes, because species showing recurrent fluctuations in their population sizes are unlikely to be at mutation–drift–migration equilibrium. This difficulty might be circumvented, at least partly, by using comparative approaches either on temporal samples of a population set collected during a period of variation in population size and/or migration rate, or on sampled population sets with contrasting historical patterns of demographic fluctuations. To our knowledge, such comparative approaches have been rarely attempted (but see Berthier *et al*. 2006 for a temporal microsatellite survey).

The migratory locust, *Locusta migratoria*, provides an ideal biological model to compare the dynamics of populations that vary in their historical frequency and extent of demographic fluctuation. *L. migratoria* is an agricultural pest of major importance in large areas of the Ancient World. Usually, the species exists at low densities in the solitarious form, characterized by relative cryptic and scattered individuals. However, at irregular intervals, it displays vast increases of population density, with actively aggregating and swarming individuals typical of the high-density gregarious form (Uvarov 1966). Such widespread outbreak events are frequently reported in large areas of the species range (hereafter referred as outbreaking areas); but records are rare or even absent in other parts of its range, in particular Western Europe (non-outbreaking areas; COPR 1982 and see the figure 1 of Chapuis *et al*. 2008). A comparison of the levels of genetic diversity and differentiation in solitarious populations, in both historically outbreaking and non-outbreaking areas, may provide important insights into the effects of outbreak events on population dynamics and structure in locusts. First, a populational homogenizing effect of outbreak events could be inferred if geographical sets of outbreaking populations are genetically less structured than sets of non-outbreaking populations (both sets being sampled at similar geographical scales). Second, in populations recurrently fluctuating in size, the long-term effective size, which determines the overall amount of genetic drift, is expected to approximately correspond to the harmonic mean size over time and should thus be closer to the size during the remission period than during the outbreak period (Motro & Thomson 1982). Consequently, if the population size of solitarious populations is large/small, we expect similar high/low genetic diversity in historically outbreaking or non-outbreaking areas.

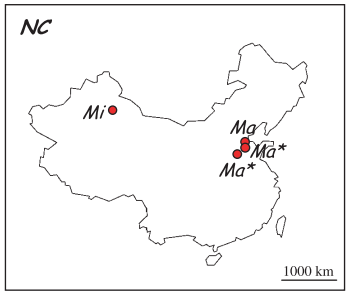
So far, two studies have provided valuable insights into this question, but have not led to any firm conclusions. For the desert locust, *Schistocerca gregaria*, Ibrahim *et al*. (2000) carried out a genetic survey in solitarious populations, sampled in Eritrea 4 years after the last outbreak event and based on a single nuclear DNA marker. This study revealed large genetic diversity within populations and substantial genetic variation among populations, despite the homogenizing potential of outbreak events. A number of hypotheses were presented to explain the latter pattern including (i) geographical isolation between breeding sites during outbreak and remission periods, (ii) failure of gregarious populations to establish at the end of the outbreak event, and (iii) the effect of repeated population extinction/recolonization events during the remission/outbreak periods (Ibrahim 2001; Ibrahim *et al*. 2000). However, the absence of comparison with genetic variation in gregarious populations in this study precluded clear inferences on the potential homogenizing effect of outbreak events on genetic variation.

Chapuis *et al*. (2008) found some indirect evidence of a homogenizing effect of outbreak events on the *L. migratoria* population structure, by using a worldwide sampling scheme. The authors used the hierarchical Bayesian method of Foll & Gaggiotti (2006) that estimates F_{ST} values as migration–drift factors for each local population and relates them to ecological factors (*Outbreaking* and *Insularity* in the case study) using a general linear model (GLM)-based approach. Mode estimates of the GLM regression coefficients indicated a moderate increase of gene flow in outbreaking populations, with a decrease of local F_{ST} values of one-third and one-half in continents and islands, respectively. However, several lines of evidence suggested that the outbreak effect may have been considerably underrated due to various confounding biogeographical factors found to also shape genetic variation in *L. migratoria*, at least over large geographical scales. A sampling scheme at a regional scale of different population sets with contrasting historical outbreak patterns would allow a more straightforward assessment of the effect of outbreak events on genetic variation.

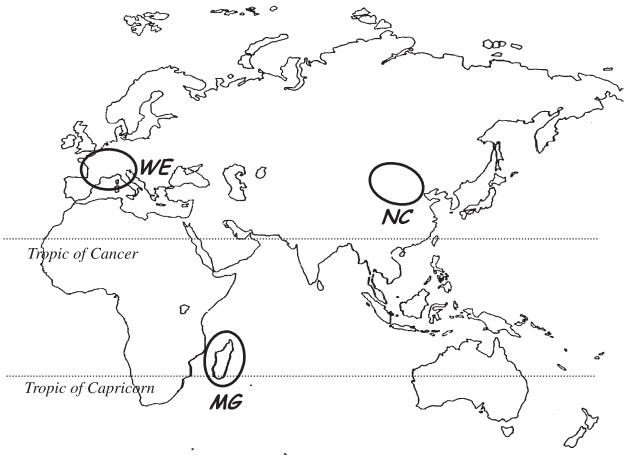
In this article, we compared the genetic variation within and among populations at 14 microsatellite markers for 24 *L. migratoria* solitarious populations located in three different geographical areas with contrasting historical patterns of outbreak events. Our comparative survey allowed useful inferences on the dynamics and structure of historically non-outbreaking and outbreaking populations of this pest species.

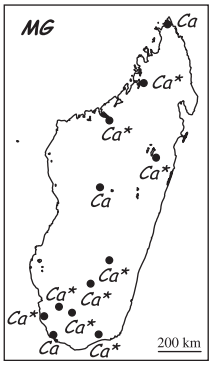


Outbreak pattern: uncommon and local
Ecological characteristics:
1–2 generations per year, absence of seasonal migration



Outbreak pattern: frequent and intense
Ecological characteristics:
1–2 generations per year, absence of seasonal migration





Outbreak pattern: frequent and intense
Ecological characteristics: 4 generations per year, presence of a seasonal migration

Fig. 1 Geographical origins, taxonomical units, and genetic clustering of sampled populations of the migratory locust. *, sites specifically sampled for this study; *WE*, Western Europe; *MG*, Madagascar; *NC*, Northern China; *Ca*, *L. m. capito*; *Ci*, *L. m. cinaerescens*; *Ga*, *L. m. gallica*; *Ma*, *L. m. manilensis*; *Mi*, *L. m. migratoria*; *Pa*, 'Palavas' form. Sampled sites with similar colours belong to the same genetic cluster as assessed by the Bayesian clustering method of Corander *et al*. (2003).

Materials and methods

Field collection and sampling design

From the autumn of 2001 through to the autumn of 2004, we collected a total of 24 population samples (19 to 31 individuals per sample) distributed in three different areas: 8 populations in Western Europe (hereafter referred to as *WE* populations), 12 populations in Madagascar (*MG*), and 4 populations in Northern China (*NC*; Fig. 1). *WE*, *MG*, and *NC* sampled areas were relatively similar in size, with a maximal distance between two samples of 1046 km for Western Europe, 1533 km for Madagascar, and 2503 km for Northern China. Nine of the 24 collected population samples had been previously analysed by Chapuis *et al*. (2008). The other 15 geographical sites were specifically sampled for this study. Chapuis *et al*. (2008) showed a poor congruence between genetic clustering and traditional *Locusta migratoria* subspecies taxonomy. However, hereafter we will occasionally refer to the traditional subspecies denomination for labelling convenience (see also Fig. 1).

The three areas studied are characterized by contrasting historical patterns of outbreak events. In *WE*, only one outbreak event, that probably originated from the western coast of the Black Sea in the 14th century (Waloff 1940), and some rare, low intensity local outbreaks have been reported (region of Naples, Italy, in 1936; Jannone 1947; region of Bordeaux, France in 1946–1948; Glize 1996). The *WE* samples, which encompass populations from the morphometric taxa *L. m. cinerascens*, *L. m. migratoria*, *L. m. gallica* and 'Palavas'

form, were hence considered as representative of historically non-outbreaking populations. In *MG*, five widespread outbreaks and two upsurges controlled by insecticides, have been reported during the last century (Randriatmanantsoa 1998). In *NC*, 14 widespread outbreaks were recorded between 1905 and 1959 in the Hebei region (Zhang & Li 1999). Since the latter date, pesticides have been able to contain outbreak events, with the exception of the 1994 and 1998 outbreak events. Locust outbreaks have also been recurrently recorded in the Xin Jiang region (Chen 1991; Tanaka & Zhu 2005). The population samples from *MG*, which belong to the taxon *L. m. capito*, and from *NC*, which belong to the taxa *L. m. manilensis* and *L. m. migratoria*, were thus both considered as representative of frequently outbreaking populations. Malagasy and Chinese populations were sampled from one to 6 years after the last recorded outbreak event.

Our sampling is in a sense imbalanced since we lack a second non-outbreaking population area. Unfortunately, *L. migratoria* populations displaying rare and local outbreaks are localized in Western Europe except for marginal populations from small isolated islands (COPR 1982 and see Fig. 1 in Chapuis *et al*. 2008). It is worth noting that potential confounding ecological factors, such as number of generations, presence or absence of seasonal migration, or island vs. mainland area, were evenly distributed across the three studied areas. Indeed, the Malagasy inter-tropical environment is favourable to many generations per year (four generations per year), no egg diapause, and seasonal migration (Lecoq 1975), whereas the European and Chinese temperate zones are associated with fewer generations per year (one to two generations per year), facultative egg diapause and absence of seasonal migration (Roubaud 1947 and Ma 1958).

Microsatellite genotyping and null alleles

We genotyped these 24 population samples at the 14 microsatellite loci described in Chapuis *et al*. (2005, 2008). We tested for and estimated the null allele prevalence as previously described in Chapuis *et al*. (2008). A large excess of significant departures from HWE (i.e. heterozygote deficiency) were observed in 190 of the 336 single-locus exact tests after false discovery rate correction (Benjamin & Hochberg 1995). Specimens that failed to amplify at some loci did not yield polymerase chain reaction (PCR) products after two or three PCR attempts, whereas the same DNA samples were successfully amplified at other loci. This strongly suggests the presence of null alleles for most loci and populations. Accordingly, Micro-Checker (Van Oosterhout *et al*. 2004) showed that the general excess of homozygotes is distributed across most allele size classes. Estimated frequencies of null alleles per locus per population ranged from 0 to 0.753, with frequencies averaged over loci varying from 0.11 to 0.24 among populations (see Table S1, Supporting Information for more details).

Statistical treatment of data

We applied a Bayesian analysis implemented in the program BAPS 3.1 (Corander *et al*. 2003) for estimating substructure in the *WE*, *MG* and *NC* areas separately. The method determines clusters of population samples minimizing Hardy–Weinberg and linkage disequilibria within the clusters and treating the number of clusters as an unknown parameter. This method has been shown to determine the uppermost level of population structure, grouping together panmictic populations that exchange migrants at a high level, (Waples & Gaggiotti 2006; Chapuis *et al*. 2008). The BAPS treatment allowed us identifying population sets homogeneous for dispersal patterns. This allowed more sensible comparisons of genetic variation among population sets (see results section). Because the simulation study of Chapuis *et al*. (2008) has shown that BAPS better discriminates gene pools in the presence of null alleles (at least with intermediate null allele frequencies), while not accepting more gene pools than there are in reality, we used the raw microsatellite data set without any correction for null alleles to infer the number of population clusters in *L. migratoria*.

With regards to genetic variation within population, it is worth noting that computer simulations performed on five statistics summarizing genetic diversity within populations [i.e. two measures of allelic diversity, the observed and expected heterozygosities of Nei (1987), and allele size variance in base pairs] have shown that the presence of null alleles led to an underestimation of all such statistics, but that this bias was particularly low for the expected heterozygosity, especially for high levels of genetic diversity, such as those observed within *L. migratoria* populations (Chapuis *et al*. 2008). Therefore, we focused the computation in the present study on the mean expected heterozygosity ($\bar{H}_E$) of each population set determined by the BAPS clustering method. Confidence intervals for $\bar{H}_E$ were calculated using the program FSTAT (Goudet 1995) from 10 000 replicates of a bootstrap resampling procedure over loci.

Within each population set, genotypic differentiation over all loci and populations was tested using Fisher exact tests (GenePop 3.3; Raymond & Rousset 1995). The level of differentiation between populations was quantified by computing Weir (1996) estimator of F_{ST} across all populations of a same set. We used the so-called *ENA* method to efficiently correct for the positive bias induced by the presence of null alleles on F_{ST} estimation (Chapuis & Estoup 2007). Unbiased $F_{ST}^{\{ENA\}}$ values and their confidence intervals, calculated using 10 000 replicates of a bootstrap resampling procedure over populations and loci, were computed using the package FreeNA (http://www1.montpellier.inra.fr/URLB/).

Table 1 Comparison of genetic variation among population sets. (a) Estimated values of $\bar{H}_E$ and $F_{ST}^{\{ENA\}}$ for the different population sets. 95% confidence intervals computed by bootstrap resampling over loci are given between square brackets. (b) Results of Wilcoxon signed rank tests (*P*-values). Significant deviations from the null expectation of no difference between population sets ($P < 0.05$) are shown in bold characters. $\bar{H}_E$, expected heterozygosities averaged over loci and populations (Nei 1987); $F_{ST}^{\{ENA\}}$, Weir's (1996) F_{ST} computed across loci and populations taking into account the presence of null alleles (Chapuis & Estoup 2007); *WE-I*, group of isolated populations in Western Europe; *WE-C*, group of connected populations in Western Europe; *MG*, Madagascar; *NC*, Northern China.

(a)

	Outbreaking population sets		Non-outbreaking population sets	
	MG	*NC*	*WE-I*	*WE-C*
$\bar{H}_E$	0.83 [0.77–0.91]	0.83 [0.74–0.91]	0.82 [0.78–0.85]	0.87 [0.82–0.91]
$F_{ST}^{\{ENA\}}$	0.002 [0.000–0.004]	0.005 [0.002–0.026]	0.058 [0.047–0.071]	0.021 [0.015–0.027]

(b)

	Pair of non-outbreaking population sets	Pair of outbreaking population sets	Pairs of population sets with heterogeneous outbreak patterns			
	WE-I vs. *WE-C*	*MG* vs. *NC*	*WE-I* vs. *MG*	*WE-I* vs. *NC*	*WE-C* vs. *MG*	*WE-C* vs. *NC*
$\bar{H}_E$	**0.003**	0.553	0.426	0.119	0.296	0.078
$F_{ST}^{\{ENA\}}$	**0.001**	0.104	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

We used Wilcoxon signed-rank tests applied on the 14 single locus values of the statistics of interest (H_E averaged over all populations of a given set and global $F_{ST}^{\{ENA\}}$ across all populations of a given set) to determine whether genetic diversity within populations and genetic differentiation between populations differed significantly between the compared population sets.

We could not estimate migration rates within the *WE*, *MG* and *NC* population sets using coalescent-based methods such as BayesAss (Wilson & Rannala 2003) or Migrate (Beerli & Felsenstein 2001; Beerli 2006). We indeed had to deal with serious problems of MCMC convergence that might be due to the high levels of gene flow in our population systems (Wilson & Rannala 2003) and/or to the presence of null alleles. It is worth pointing out here that the effect of null alleles on such Bayesian or maximum-likelihood approaches is unknown, although it might underestimate migration rates as suggested by the increase of uncorrected F_{ST} indexes due to the presence of null alleles (Chapuis & Estoup 2007).

Results

The BAPS test provided the highest probability for a single population cluster in the *MG* area and in the *NC* area, and for structuring into five population clusters in the *WE* area (Fig. 1; probabilities of the model = 1). The *WE* area includes two sets of populations characterized by different patterns of genetic structure: a set of populations distributed in the southern part of Western Europe connected by high levels of gene flow (i.e. the cluster with the four sampled populations belonging to *L. m. cinerascens*; hereafter referred to as the *WE-C* set), and a set of populations distributed all over the sampled area, either isolated or exchanging migrants at a low level (i.e. the four genetic clusters with a single population; hereafter referred to as the *WE-I* set). We will hereafter compare genetic variation, within and among populations, between four population sets: the historically non-outbreaking *WE-I* and *WE-C* population sets and the historically outbreaking *MG* and *NC* population sets.

The level of within-population genetic diversity — measured by the expected heterozygosity — is given for each of four population sets in Table 1a (see Table S1 for details on each of 24 studied populations). The two outbreaking population sets *MG* and *NC*, although located in regions with different ecological features, had similarly high levels of within-population genetic variation (Table 1; $P = 0.553$). In the non-outbreaking *WE* area, the within-population genetic variation was significantly lower for populations of the set *WE-I* than for the set *WE-C* (Table 1; $P = 0.003$). No significant differences were detected in any of the pairwise comparisons concerning population sets with different outbreak patterns (i.e. *WE-I* and *WE-C* vs. *MG* and *NC*; Table 1; $0.078 \leq P \leq 0.426$). Altogether, these results indicated that genetic diversities were remarkably similar (and high) within non-outbreaking and outbreaking populations.

Global genetic differentiation between population samples was highly significant within each population set ($P \leq 10^{-4}$) except for the *MG* population set ($P = 0.171$). In the *WE* area, the level of genetic structure was about threefold

greater for the *WE-I* populations than for the *WE-C* populations (global $F_{ST}^{\{ENA\}}$ estimates of 0.058 and 0.021, respectively, $P = 0.001$; Table 1). Both *MG* and *NC* outbreaking population sets, although located in regions with different ecological features, had nonsignificantly different low levels of genetic structure (global $F_{ST}^{\{ENA\}}$ estimates of 0.002 and 0.005, respectively, $P = 0.104$; Table 1). Global $F_{ST}^{\{ENA\}}$ estimates were fourfold to 30-fold lower for the outbreaking population sets (*MG* and *NC*) than for the non-outbreaking population sets (*WE-I* and *WE-C*) ($P < 0.0001$ for all pairs of population sets; Table 1b). All pairwise $F_{ST}^{\{ENA\}}$ estimates of both *MG* and *NC* outbreaking population sets (ranges of 0–0.011 for both sets) were lower than those of both *WE-I* and *WE-C* non-outbreaking population sets (ranges of 0.042–0.066 and 0.013–0.028, respectively). Altogether, these results indicate that populations in areas with frequent and intense outbreak events are substantially less structured than populations in areas with uncommon and local outbreak events.

Discussion

New insights into outbreak dynamics

We found similar high genetic diversity within non-outbreaking and outbreaking populations of the migratory locust. Genetic diversity within outbreaking populations was not strongly affected by recurrent increases of population sizes during outbreaking periods. This result is consistent with the theoretical prediction that the long-term effective size in populations recurrently fluctuating in their size is expected to approximately correspond to the harmonic mean size over time and should thus be closer to the size during the remission period than to that during the outbreak period (Motro & Thomson 1982). It is worth pointing out that the relatively large amount of genetic variation observed within all populations indicates that solitarious locusts persist in populations of large effective size in both outbreaking and non-outbreaking areas. Accordingly, the densities of solitarious migratory locusts were estimated to vary from 1 to 1000 individuals per hectare with seasons, years, and localities in Madagascar (Lecoq 1975). Densities of 1 to 200 individuals per hectare have locally been observed in the non-outbreaking French Mediterranean coast (Remaudière 1948a, personal communication).

The level of population differentiation, as measured by $F_{ST}^{\{ENA\}}$, was fourfold to 30-fold larger among historically non-outbreaking Western European populations than among the outbreaking Malagasy and Chinese populations. The lower level of genetic differentiation observed among populations from outbreaking areas can be accounted for by higher gene flow among populations and/or larger effective population sizes (less genetic drift). We found estimates of genetic diversity to be similar among non-outbreaking and outbreaking populations, suggesting similar harmonic means of effective population size. Therefore, our microsatellite survey indicates that the observed difference in genetic differentiation is due to substantially higher gene flow among outbreaking populations.

Our results do unambiguously show a severe homogenizing effect of outbreak events in *Locusta migratoria*. Chapuis *et al*. (2008) found indirect support for a moderate increase of gene flow in outbreaking populations, by using a worldwide sampling scheme and the hierarchical Bayesian method of Foll & Gaggiotti (2006). However, the efficiency of the latter method for correctly identifying the *Outbreaking* effect depended on additional structuring factors. In particular, the authors had to include a second factor in their model, *Insularity*, to account for the barrier effect to dispersal of water masses. The worldwide genetic survey of Chapuis *et al*. (2008) also showed evidence of additional structuring factors such as high mountain ranges, as well as some imprints of long-distance colonization events. Such environmental factors and historical events are difficult to integrate in the model of genetic structure developed by Foll & Gaggiotti (2006). By using a comparative approach based on a regional sampling scale, we could minimize the above drawbacks and provide a more straightforward assessment of the actual strength of the effect of outbreak events on neutral genetic variation.

The more intensive gene flow among populations of outbreaking areas may be the result of demographic or behavioural factors. First, as outbreaking populations experience recurrent demographic flushes, they may recurrently produce a larger number of effective migrants than non-outbreaking populations. Second, the high density of individuals during outbreak periods is likely to represent a strong demographic pressure for migration, which may result in an increase of the rate and/or distance of effective migration in gregarious populations. In agreement with the latter point, important changes in traits of migratory behaviour have been observed with the gregarious phase in locusts. First, the actively aggregative forms have been interpreted as adaptations for long-distance migration at least at the nymph stage (Ellis 1953). Second, it has been observed that gregarious adults, which fly during the day, frequently overfly habitats that solitarious adults, which fly at night, cannot reach because of low night temperatures (Uvarov 1966). The high propensity for migration of gregarious individuals is illustrated by recurrent reports of swarms moving downwind 100 km or more in 9 or 10 hours of daytime (FAO 1994).

The strong homogenizing effect of recent outbreak events and the large amount of genetic variation within solitarious populations of *L. migratoria* render implausible, at least for this species, the hypothesis of genetic isolation of solitarious and gregarious forms within a given area, as well as that of drastic reductions of population viabilities at the time of outbreak declines (Ibrahim *et al*. 2000). Ibrahim *et al*. (2000)

did not find evidence for a homogenizing effect of outbreak events on genetic variation in *Schistocerca gregaria*. *S. gregaria* and *L. migratoria* might differ in the populational characteristics of their gregarious and/or solitarious forms. Accordingly, it is sometimes mentioned that solitarious *S. gregaria* exist as small populations in patchy environments and are particularly prone to extinction because of climatic events (Chapman 1976; Ibrahim *et al*. 2000). The homogenizing effect of outbreak events on genetic variation of *S. gregaria* may have also been underrated by the absence of comparison with genetic variation in non-outbreaking areas.

Implications for preventive control

For *L. migratoria*, as for many other pest species, acting early in the outbreak process has become essential to hamper small upsurges, to limit agricultural losses and to safeguard the environment (i.e. limit insecticide spraying) (Lecoq 2001). Such preventive strategy is based on controlling first crowdings of transient locusts rather than the vast and highly mobile bands or swarms of gregarious locusts. This strategy requires determining the origin of nascent outbreak populations and their subsequent movements, and this requirement highlights the benefits of using genetic markers (e.g. see 2006–2010 research priorities of the Australian Plague Locust Commission: http://www.affa.gov.au/aplc). To what extent would genetic monitoring be useful for the surveying and preventive management of locust pests depends on our statistical ability to detect migrants and infer their origin. The traditional genetic approaches for inferring sources and routes of invasive processes, using *F*-statistics (e.g. Weir 1996) or assignment statistics (e.g. Rannala & Mountain 1997; Paetkau *et al*. 2004), lack resolution when genetic differentiation between populations is low (e.g. Roeder *et al*. 2001), as found in the present study in historically outbreaking areas of *L. migratoria*. An alternative promising approach relies on the use of genetic linkage disequilibrium information present in multilocus genotypes, as done by the Bayesian method developed recently by Gaggiotti *et al*. (2002, 2004). This method estimates the proportion of individuals that different source populations contribute to a genetic mixture and complements the genetic information with demographic and geographical distance data. It has been shown to be relatively insensitive to the level of genetic differentiation (Gaggiotti *et al*. 2004). Computer simulation studies are, however, needed to assess the power of the latter method in the specific context of historically outbreaking areas of *L. migratoria* characterized by large effective sizes for both source populations and swarms.

Population structure in Western Europe

We found that the sampled Western European populations comprised a group of populations that belongs to the subspecies *L. m. cinerascens* located in southern part of Western Europe (*WE-C*) and a group of populations distributed all over the sampled area (*WE-I*) with lower genetic diversity and higher levels of population differentiation. This pattern could be explained by a smaller effective population size of *WE-I* populations, which could be due to a lower carrying capacity of their habitat. Indeed, Northern and Western Europe correspond to limits of the species range. For instance, the population of *L. m. migratoria* sampled here is the northernmost reported population in Western Europe (P. Meunier, Musée d'Histoire Naturelle du Mans, France, personal communication). In contrast, the ecological conditions of the *WE-C* population set cannot be viewed as marginal. For instance, they are comparable to those where *NC* populations were sampled (see Materials and methods section). Interestingly enough, a lower carrying capacity of habitats is unlikely to hold also for the *WE-I* populations belonging to the genetically differentiated 'Palavas' form. This form is found in a small area that comprises Mediterranean lagoon ecosystems that offer wet areas particularly favourable to the migratory locust (Remaudière 1948b).

The lower genetic diversity and higher level of population differentiation of *WE-I* populations of Western Europe could be also explained by a lower level of gene flow due to geographical isolation. Although this might be the case for the Northern and Western European populations, this is unlikely for the Palavas populations since the latter are located on the Mediterranean coast along which populations of the subspecies *L. m. cinerascens* are commonly found (Fig. 1). The pre- and/or post-mating mechanism(s) involved in the apparent disruption of effective gene flow of the Palavas form with neighbouring *L. m. cinerascens* populations are unknown. Adults of the 'Palavas' form are notorious for their remarkably larger size than that of their neighbours (Remaudière 1948a; personal observation). It is conceivable that premating barriers through size-assortative mating may have evolved, rendering migration between the Palavas form and *L. m. cinerascens* less effective. Assortative mating by size is common in insects (reviewed in del Castillo *et al*. 1999), and can be generated by mechanisms such as mate choice, mate availability, and mating constraints. Field and laboratory experiments on mating behaviour are needed to test this hypothesis.

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MPC is currently using molecular and evolutionary ecology approaches to understand the evolution of population outbreaks and extreme density-dependent phenotypic plasticity in locust species. AL is a molecular biology technician working on various insect and rodent populations. YM's main interests are host–parasite interactions and the evolution of life-history traits, reproductive system and dispersal. ML is currently interested in understanding the population dynamics and underlying mechanisms of outbreak formation of various Orthopteran pest species, including the migratory locust and the red locust in Madagascar, as well as the Senegalese grasshopper in Niger. The focus of his work is also on improving the preventive control strategies of the desert locust in Western Africa. AF is focusing on ecology of the Acrididae using Remote Sensing and GIS technologies to improve forecasting and pest management methods. AE's current research focuses mainly on the evolutionary biology of non-equilibrium populations with a particular interest in invading species.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Mean heterozygosities and null allele frequency estimation for each of 24 studied *Locusta migratoria* populations. *N*, sample sizes in number of diploid individuals, H_O, mean observed heterozygosities; H_E, mean expected heterozygosities (Nei 1987); $\hat{r}_D$, mean null allele frequencies computed across loci with the method of Dempster *et al*. (1977) on the original genotype data sets; *WE-I*, group of isolated populations in Western Europe; *WE-C*, group of connected populations in Western Europe; *MG*, Madagascar; *NC*, Northern China, see 'Results' section for more details.

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