Frequent colony relocations do not result in effective dispersal in the gypsy ant *Aphaenogaster senilis*

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Dispersal is an important step in animal's life cycle, one consequence of which is reducing local mate and resource competition. Dispersal is often achieved during one unique special movement, from the birthplace to a new appropriate area where to settle and reproduce. However, in species in which this special movement is limited by life history tradeoffs, we may expect dispersal to be promoted also by routine movements occurring throughout the animal's life and stimulated by other activities like foraging or the search of nesting conditions. Here we employ a multidisciplinary approach consisting of computer simulations, mark–recapture and genetic data to better understand the role of colony relocations as dispersal strategy in the gypsy ant Aphaenogaster senilis. Contrary to expectations, our results show that colony relocations do not result in effective dispersal as evidenced by mark–recapture and genetic data. Furthermore, simulations showed that successive colony relocations did not follow a constant direction, but occurred either in a randomly changing direction or followed a circular trajectory, indicating limited effective dispersal. We also found a general lack of inbreeding and significant population viscosity between neighbouring colonies suggesting that relocations may act as a balancing strategy between these two processes. We discuss the results in terms of their evolutionary and ecological significance, and highlight future directions of research towards the understanding of dispersal strategies in colonial species.

Dispersal is a key process in animals with major implications in ecology, evolution and conservation (Clobert et al. 2001, Wang and Smith 2002). Both theoretical and empirical studies have largely unravel the importance of dispersal processes in inbreeding avoidance (Waser et al. 1986, Bollinger et al. 1993), kin competition (Hamilton and May 1977, Kisdi 2004, Ronce and Promislow 2010) and gene flow among geographically distant populations (Fahrig and Merriam 1985, Hansson 1991, Galarza et al. 2009). Conceptually, dispersal is often viewed as the spread of individuals from their natal site to settle and reproduce in new areas (Johnson 1969, Greenwood and Harvey 1982, Hawkes 2009). Given its importance, dispersal can derive from a special movement that evolved specifically to allow the displacement of individuals away from their natal site, generally early in their life cycle. Such special movements are well exemplified in species with important dispersal polymorphism. In other species, however, dispersal results from routine movements associated to other activities like foraging or exploration leading to effective dispersal if reproduction takes place in different locations (Van Dyck and Baguette 2005).

Ants are interesting models in population ecology because different species have evolved various dispersal modes in relation with colony foundation (Hölldobler and Wilson 1990, Bourke and Franks 1995). On the one hand, the virgin queens of many species bear long wings activated by a hypertrophied thoracic musculature that allow them flying over relatively long distances (Peeters and Ito 2001). After mating during a nuptial flight, they shed their wings and almost immediately start searching for a nest location to initiate a new colony by themselves. While independent colony foundation exposes queens to important mortality rate due to predation and competition with established colonies (Gordon and Kulig 1988, Wiernasz and Cole 1995, 2003, Adams and Tschinkel 2001, Boulay et al. 2007), it is thought to guarantee enough gene flow to prevent isolation by distance (Helmkampf et al. 2008). On the other hand, some ant species found new colonies by colony fission. Here, queens either have small non-functional wings or are completely wingless (Molet and Peeters 2006, Molet et al. 2008, Amor et al. 2011). They leave their mother nest accompanied by a group of workers to found a new colony at a walking distance. This strategy increases queen survival during colony foundation but, at the same time, it limits the distance at which the new colony can establish (Pamilo 1991). This strategy is expected to translate into high genetic structuring and limited gene flow, even within short distances

(Seppä and Pamilo 1995, Giraud et al. 2000, Clémencet et al. 2005, Berghoff et al. 2008) resulting in a high relatedness among neighboring colonies (population viscosity), which in turn, may give rise to local resource competition (Wilson et al. 1992, West et al. 2001).

Although some ant species rarely abandon their nest during decades (e.g. *Pogonomyrmex occidentalis*, Keeler 1988), others perform frequent colony relocations. Extreme nomadism occurs in army ants of the Dorylinae, Leptanillinae and Ecitoninae subfamilies, in which it is mainly tied to predation. These species do not build a structured nest but only form temporal bivouacs where an enormous mass of workers aggregate around the queen. Beside these species with a particular lifestyle, colony relocations also occur with a relatively high frequency in several genera, including *Solenopsis* (Fuller et al. 1984, Tschinkel 2006) *Pheidole* (Droual 1984), *Temnothorax*, formerly *Leptothorax* (Dornhaus et al. 2004) and *Aphaenogaster* (Smallwood 1982, McGlynn et al. 2004, Boulay et al. 2010). The causes of colony relocations can be very diverse, however. Some species relocate their colony after an external perturbation such as flooding (Tschinkel 2006) or when microclimatic conditions change (Smallwood 1982, Gibb and Houchuli 2003, Heller and Gordon 2006). For other species, colony relocation may also occur as a response to an attack by competitors (Cerdá and Retana 1998, Brown 1999, Dahbi et al. 2008) predators (LaMon and Topoff 1981, Droual 1984) to reduce the parasitic load (Droual 1984, Gordon 1992, McGlynn et al. 2004) or to be near available food resources (Mabelis 1979, Maschwitz and Hänel 1985).

Even though the proximate causes of colony relocation are relatively well understood in several ant species, the genetic consequences of such movements at the population level remain widely unexplored, particularly for fission-performing species. For example, if on the course of several relocations, each colony tends to keep a relatively constant direction, we may expect the distance between related colonies to increase progressively, and thus reducing population viscosity. By contrast, if colonies tend to repeatedly use the same set of nests, forming a kind of circular movement, relocations may have little consequences on the population genetic structure. Such nest re-utilization after a few relocations (i.e. serial monodomy), has recently been described in the gypsy ant *Aphaenogaster araneoides* (McGlynn 2010). In the present study, we examine the effects that colony relocations have at the population level in delineating genetic structure and population viscosity in another gipsy ant *A. senilis*, which founds new colonies by fission. Specifically, we determined if relocations occur randomly within the landscape, and if they have an effect in population viscosity. For this purpose, we monitored colony movements through a mark–recapture approach combined with population genetic analyses and computer simulations. In addition, we document the process of colony fission, which is poorly understood in ants.

Material and methods

Model species and study sites

Aphaenogaster senilis is a common, omnivorous species distributed along the Mediterranean basin from southern France to Morocco. Colonies are strictly monogynous and monoandrous and contain between 200 and 3000 workers (mean ± SE: 1260 ± 69; Boulay et al. 2007) the region surrounding the Doñana National Park (southern Spain) *A. senilis* is particularly abundant in sandy areas. For the purpose of this study, four sites were selected, inside the Reserva Biológica de Doñana (RBD, hereafter), namely *Comedero*, *Beles*, *Jaulon* and *Visita*. The distance between these sites ranged between 4 and 6 km. A fifth site (*Algaida*) located in San Lucar de Barrameda (30 km southeast from RBD sites) was also selected. Vegetation at the five sites mostly consists of open scrublands (*Halimium halimifolium*, *Halimium commutatum*, *Stauracanthus genistoides* and *Lavandula stoechas*). *Comedero* presents the same shrub species along with sparse pine trees (*Pinus pinea*) and savin juniper trees *Juniperus sabina*. *Algaida* also presents numerous mastic shrubs (*Pistacia lentiscus*).

Colony monitoring by mark–recapture

In order to study the process of colony relocation, 21 focal colonies were excavated between January and March 2006 at *Beles*, *Jaulon* and *Algaida*. Focal colonies were chosen so they were separated by at least 10 m. Once in the lab, they were cooled down 10–30 min on ice at 0°C to mark all the workers and the queen with a dot of paint (Mitsubishi pencil) on the abdomen. Different colours were used for different colonies originating from the same site. All the marked workers, the queen and the brood were then released in the field at the spot of capture no later than a week after nest excavation.

To ensure that paint marks remained visible over time, we conducted a preliminary pilot study in spring 2005, when 400 workers from an excavated nest were marked with two dots of paint on the abdomen and on the thorax and were then returned to the same place. After a month, we recaptured 86% of the originally marked ants, 98% of them still showing both marks and 2% showing only one mark. The much higher abundance of ants with two marks clearly suggests that those ants captured without marks were mostly new ants not marked in the first place. This indicates that the paint marks have a relatively long life and that the progressive disappearance of marked ants in focal nests was mostly due to high worker mortality in late spring. Similarly, in a previous study (Boulay et al. 2009), we have determined the production cycle of both queen and workers through a six-year follow-up of more than 300 colonies. The results showed a significant reduction in worker abundance during spring followed by a peak abundance in early summer. This suggests a worker turn-over for this species of approximately 11–13 months when the majority of worker force is replaced. Therefore, both mark endurance and worker turn-over are well within the time frame of the present study. To ensure that colony monitoring could follow up during up to ten months that this study lasted, we repeatedly marked unmarked workers collected at the entrance of focal nests.

The location of marked colonies was then monitored once a week until they were lost and no later than November 2006. Colony location was determined by carefully scanning an area of 10 m around the last location. The area was scrutinized between one or two people depending on the complexity or the vegetation for at least 20 min. *Aphaenogaster senilis* nests

are often ornamented with flower petals and can be clearly seen (Supplementary material Appendix A1 Fig. A1). When a marked worker was detected, she was hand fed a small piece of biscuit and followed back to her nest. If the colony had relocated, we measured the distance from last location and the distance to the origin (e.g. the first colony location after its release). Colonies lost during the course of the experiment were assumed to have moved at least 10 m. Nest half-life (Nhl), which refers to the number of days for half of the colony to relocate, was calculated according to King and Sallee (1956) as following:

$$Nhl = R \times \ln(2)$$

where R is the average residence time (the average number of days between two relocations).

The area around the last nest location was also checked for possible fission of the focal colonies. When a fission was confirmed by the presence of same-marked ants in two nests, both the mother and daughter nests were excavated. They were brought to the lab to count the workers but they were not released in the field and the monitoring of their trajectory was stopped.

We tested whether the probability of colony relocation was influenced by previous relocations by fitting a generalized linear model (GLM) using the glmer command for R (The R Core Team 2010) with the binomial error distribution and logit link function. The probability of relocation during the current week (Preloc) was the response variable and the week (Wk), having relocated or not the previous week (RelocPrev) was considered as fixed factors in the full model. The sampling site (Sp) and the Colony (Col) were considered random factors. Wk was also included in the random part of the model to account for temporal variations within colonies. The sign of the RelocPrev estimate indicated whether the probability of relocating increased or decreased after a first relocation. We also fitted a second generalized linear model with the log-transformed relocation distance (Dreloc) as a response variable, Wk and Sp as fixed factors and Col as random factor. Backward model selection was conducted by comparing Akaike information criteria (AIC) using log likelihood ratio tests until all non-significant factors were removed. The scripts for both models are given in the Supplementary material Appendix A1 Table A3. Finally we tested the correlation between relocation distance and colony size (number of workers at 1st capture) by means of simple linear model.

Computer simulations

To identify possible patterns described by colonies after several relocations, we compared real relocations with those expected under a random-walk. First, we tested whether ant colonies showed any directionality over successive relocations. The real data was compared with random-walks simulated with NetLogo ver. 4.0.4 (code available from authors upon request). For each real colony 1000 random trajectories were simulated using the observed number of relocations, their distances and order in which the relocations were undergone. Thus, the only aspect in which the simulations differed from the real data was on the turning angles performed by colonies from one relocation event to the next.

Then, from each simulation we extracted the two values that we also gathered from real colonies, namely the distance between the final location and the origin and the cumulated distance between each intermediate location and the origin. The statistical significance of the difference between real and simulated data was estimated as the proportion of simulations with values lower (or larger) than the real value (i.e. $p = 0.5$ means that the real value is equal to the average of 1000 simulations, and $p = 0.01$ would mean that only 1% of simulated data was larger, or lower, than the real value).

Genetic structure and population viscosity

A total of 261 adult workers were collected in 61 colonies located at *Comedero*, *Visita* and *Beles* in April 2008. At each site, we first selected four to seven colonies separated by at least 50 m. We then sampled their two or four nearest neighbours. For each colony, a mean of 3.5 workers were genotyped at six species-specific polymorphic microsatellite markers (Galarza et al. 2009). The total number of alleles per locus and colony was obtained using GENETIX ver. 4.01 (Belkhir et al. 1997). Observed and expected heterozygosities within sites were calculated using the software package Arlequin ver. 2.0 (Schneider et al. 2000). Deviation from Hardy-Weinberg expectations (HWE) and linkage disequilibrium within sampling sites were estimated according to the level of significance determined by means of 10 000 Monte Carlo iterations using GENEPOP ver. 3.4. (Raymond and Rousset 1995). For these tests, a reduced dataset was used which included one randomly selected worker from each colony. The false discovery rate (FDR) procedure (Benjamini and Hochberg 1995, Verhoeven et al. 2005) was employed to correct for possible type I errors when performing multiple tests. This procedure removes the fraction of false positives among all tests that are declared significant. For example, we set a FDR of 5% meaning that (on average) 5% of the tests declared significant are actually false positives.

The level of genetic structure was assessed by calculating the overall F_{ST} values (Weir and Cockerham 1984) including all colonies within each sampling site. Significance was obtained by 10 000 iterations executed in GENEPOP ver. 3.4. (Raymond and Rousset 1995) and FDR corrections were applied for multiple tests. Similarly, a two-way hierarchal analysis of molecular variance (AMOVA) was performed in Arlequin ver. 2.0 (Schneider et al. 2000) to evaluate whether genetic variation was greater among or within sampling sites.

In order to determine the degree of population viscosity, we first calculated the relatedness coefficient (R) between colonies (i.e. average pairwise relatedness between individuals from different colonies) using Relatedness 5.0 software (Queller and Goodnight 1989). We then performed a spatial autocorrelation analysis using GenAlEx ver. 6 (Peakall and Smouse 2006) to test whether significant relatedness (i.e. higher than random) occurs between pairs of colonies within a given distance class. We set an increment of 5 m for each distance class up to 100 m. Statistical significance for the null hypothesis of no significant relatedness was determined by creating 95% confidence intervals around R-values through 999 random permutations. Under the hypothesis of restricted effective dispersal due to colony fission, relatedness

values should decrease as a function of distance, and spatial autocorrelation should be observed at short distances only.

Results

Colony monitoring by mark–recapture

All monitored colonies relocated to some extent during the study period (Supplementary material Appendix A1 Table A1). On average, they changed 6.14 ± 0.46 (hereafter, mean $\pm$ SE) times of nest between their release in the field (starting late February 2006) and the last time they were observed (at most in November 2006). About 20% of the surveyed colonies each week were found in a new emplacement (Fig. 1). The average colony residence time was 29.0 ± 3.6 days and the nest half-life was 20.1 days. There was no linear trend in the probability of relocation throughout the experiment (Fig. 1; $F_{1,39} = 0.87$, $p = 0.358$), suggesting a low effect of our initial colony excavation upon their relocation behaviour. If this would have been the case, we would have expected a higher relocation rate just after the manipulation. Generalized linear model selection based on the AIC did not retain Wk in fixed and random factors (its removal lead to small decrease of the model AIC). This indicated that the probability of relocation did not differ between weeks, even within colonies (see Supplementary material Appendix A1 Table A3 for details of model selection). However, a colony that had relocated on one week had a higher probability to relocate again the following week (estimate: 0.43 ± 0.23; $z = 2.13$, $p = 0.033$). This was clearly demonstrated by the highly significant increase of the AIC when the factor RelocPrev was removed from the model ($\chi^2 = 14.34$, DF $= 1$, $p < 0.001$; Supplementary material Appendix A1 Table A3). The variance explained by differences between colonies was very small (variance $< 10^{-11}$) compared to the variance explained by differences between sampling sites (0.217).
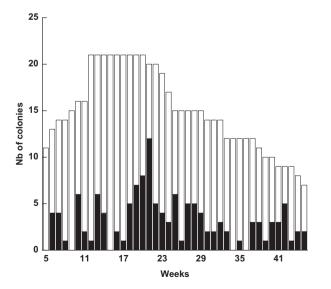
Relocation distances showed a long-tailed frequency distribution (Fig. 2) with relocations shorter than 2 m and longer than 8 m representing 50% and 19% of all relocation events, respectively. Relocation distances did not differ significantly between weeks as indicated by the non-significant variation of the AIC when this factor was removed ($\chi^2 = 3.29$, DF $= 1$, $p = 0.069$; Supplementary material Appendix A1 Table A3). As for the probability of relocation, relocation distances varied mostly between sampling sites (variance $= 0.73$) rather than between colonies within sampling sites (variance $< 10^{-3}$). Similarly, relocation distance was not significantly correlated with colony size (linear model: $F_{1,19} = 0.01$, $p = 0.88$).

Only two out of the 21 focal colonies fissioned during the course of the survey. Both fissions occurred in August 2006 at *Algaida*. In both cases the mother (marked) queen was found in a new nest located 2.3 or 2.7 m away from the old nest, which contained a single unmarked queen. These queens were surrounded by marked and unmarked workers, which confirmed the fission.

Computer simulations

Random-walk simulations indicated that successive relocations did not follow a constant direction. On the contrary, the general movement described after several relocations was either random or, in a few cases, tended to be circular, that is, colonies re-occupied several times the same nest location. Colonies moved away from their initial location as much or less than predicted by a random walk. An example of such simulations for colony no. 619 is given on Fig. 3. This colony realized eight relocations of, on average, 3.1 ± 1.2 m which led it at the end of the study to occupy a nest located more than 10 m away from its origin (black arrow). A total of 1000 simulations preserving the same relocation distances in the same order but allowing the colony to choose a random turning angle between consecutive relocations indicated that the distance between initial and final locations could range from 0.6 (the colony almost return to the origin, left end of the distribution) to 20.7 m (directional relocations, right end

Figure 1. Number of *Aphaenogaster senilis* colonies surveyed each week (of the calendar) and number of colonies that relocated (black segments).
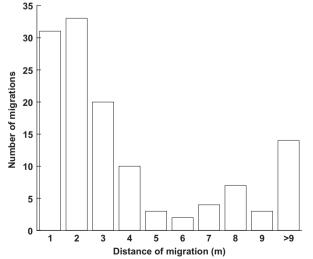

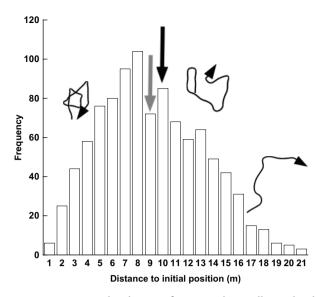Figure 2. Frequency distribution of relocation distances of the 21 focal *Aphaenogaster senilis* colonies.

Figure 3. Frequency distribution of 1000 random-walk simulated relocation distances between the initial and the last locations for colony no. 619.
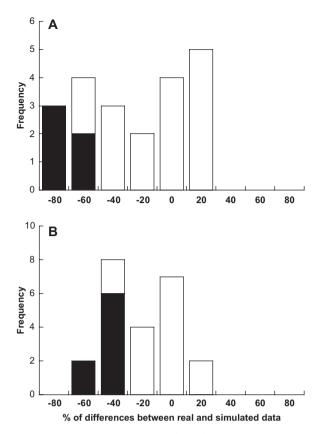

Figure 4. Comparison between real and simulated relocations. In simulations, colonies migrate the same distances than in the real data but turn in random directions before each migration. x-axis show the % of difference (e.g. + 50% means that the colony was found 3 m away from first location, but that the mean value from the simulations was 2 m, that is, $((3 - 2)/2) \times 100 = 50\%$). A value of zero means that the colony relocated as the average of the simulated random walks. Black and white bars show values departing and not departing significantly (p < 0.05) from simulated data, that is, from a random walk. (A) Distance of the colony from the first location of the colony at the end of the study period. (B) Cumulated distances of the colony from the initial colony location. Cumulated distances are calculated by the sum of all partial distances to the initial colony location each time the colony relocates.

of the distribution) with an average of 8.5 m (grey arrow). Thus, colony no. 619 moved at least 17.6% longer than what would be predicted by random walk, but this slight difference was not statistically significant (p = 0.349).

Four other colonies moved slightly further away than predicted randomly, although in no case was the difference significant. However, sixteen colonies moved less than expected randomly and for five of them the difference was significant at p < 0.05 (Fig. 4A). Ten of these colonies were seen reoccupying a nest they had left a few weeks before. Similarly, the cumulated length of the eight relocations realized by colony no. 619 was 35.1 m but the cumulated distance between each intermediate location and the origin was only 20 m. Overall, the cumulated distance between each intermediate location and the origin was significantly shorter than expected randomly for six colonies (Fig. 4B). This also refutes the hypothesis of successive relocations following a constant (linear) direction.

Genetic structure and population viscosity

The total number of alleles per locus within colonies ranged from two to three as expected for haplo-diploid monogynous-monoandrous species. No evidence of linkage disequilibrium was observed between any locus pair. Similarly, none of the probability tests for Hardy-Weinberg expectations remained significant after FDR correction for multiple tests. This suggests that no inbreeding occurs within sampling sites and the loci can be considered independent. Overall F_{ST} values within sampling sites were relatively high ranging from 0.039 in *Beles*, to 0.040 and 0.043 at *Visita* and *Comedero* respectively. This denotes a high degree of genetic structuring within the sampling sites. Similarly, the hierarchical AMOVA indicated that the majority of genetic variation occurred among colonies within sampling sites (Table 1).

Significant population viscosity was revealed by the spatial autocorrelation analysis. The results showed significant autocorrelation between relatedness values at the 0 to 5 m and at

25 to 30 m distance classes (Fig. 5). This indicates that although relatedness among neighbouring colonies within these intervals is relatively low, it is still significantly higher than expected by chance. It is important to notice, however, that a single correlogram may not reflect accurately the true non-random spatial genetic pattern. The autocorrelation largely depends on the extent of the genetic structure, the size of the distance class chosen and the associated number of samples per distance class (Peakall et al. 2003, Peakall and Smouse 2006). Thus, each sampling site could have a distinct spatial autocorrelation pattern. To account for this possible site effect, we performed independent autocorrelation analyses within each site using the same parameters as above. The results were consistent across the three sites indicating significant autocorrelation at the 0–5 and 25 to 30 distance classes (Supplementary material Appendix A1 Table A2). Nevertheless, the result for 25–30 m distance class should be taken cautiously as no pairs of samples were available in *Visita* for this interval. Therefore, the result of the autocorrelation analysis suggests

Table 1. Two-way hierarchal analyses of molecular variance (AMOVA). Significance of fixation index values obtained after 1023 permutations are given in parenthesis.

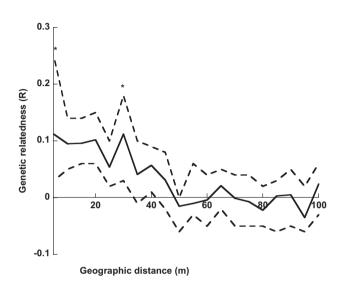| Source of variation	DF	Percentage of variation	Fixation index
Among sampling sites	2	1.79	$F_{CT} = 0.01787$ (0.00293)
Among colonies within sampling sites	58	39.51	$F_{SC} = 0.40231$ (0.0000)

Figure 5. Pairwise relatedness values (R) of *A. senilis* colonies across increasing geographic distance classes. Dotted lines indicate upper and lower 95% CI of R after 999 permutations. Significant autocorrelations are denoted with an asterisk.

a genetic-patch-size (sometimes referred to as genetic neighbourhood) of <5 m for colonies of *A. senilis*.

Discussion

The present study shows that, 1) successive colony relocations in *A. senilis* do not follow a constant (linear) direction but describe a random or circular movement; 2) they do not prevent population viscosity at a local scale, and 3) a high genetic structuring exists within the sampled area. Thus, frequent colony relocations are not a mean to increase effective dispersal in this fission performing gypsy ant.

Colony relocations

Over the six-month survey, nest occupancy had a Nhl of 20.1 days which is very similar to what was found by Smallwood (1982) for *Aphaenogaster rudis* (20.6 days) in West Virginia. All colonies relocated several times during the study, most often over a few meters, though the relocation distances varied between sampling sites. This difference of relocation distance might be due to intrinsic properties of each locality including the availability of nest sites or colony density. Future studies could test these hypotheses. Two fission events were detected during the summer 2006. In both cases, the old queen moved to another nest with 69% and 66% of the

workers while the new queen inherited the old nest and the remaining workers. This pattern is similar to what is known in other species like the honeybee *Apis mellifera* (Seeley 1997). It differs, however, from what happens in *Cataglyphis floricola* in which the queen remains in the old nest (Amor et al. 2011) or *C. cursor*, in which queens can either stay or move to a new nest (Lenoir et al. 1988, Chéron et al. 2011). In both observed cases of fission, the mother colony (which contained the old queen) settled at a very short distance from the daughter colony (2.3 and 2.7 m).

The result of our simulations indicated that colonies either moved randomly or described a circular trajectory by using several times the same nest, which suggests an opportunistic behaviour of the ants during relocation. What stimulates relocations in *A. senilis* has not been investigated in great detail yet. The lack of a clear temporal pattern (non significant difference in the probability of relocation between successive weeks) suggests that relocations might be driven by a series of factors. In a recent study, no increase in migrations was found following an experimental reduction of neighbour colony density indicating that intra-specific competition may not determine the probability of relocation in this species (Boulay et al. 2010). In *A. senilis* like in other congeneric species, sun exposure and attacks by predators and parasites were shown to stimulate nest relocation (Smallwood 1982, McGlynn et al. 2002, 2004). Other nest disturbances (including flooding, perturbations induced by large mammals and human activities) or food shortage could also trigger emigration, as in other Myrmicines (Hölldobler and Wilson 1990, Wilson et al. 1992). Insight in the decision-making rules during emigration in other species indicates that after a sudden perturbation, scouts start to explore the environment in all directions until the most suitable nest site has been discovered (Franks et al. 2003). Scouts may also use latent knowledge about potential nest locations around their current nest (Franks et al. 2007). Then, recruitment and social transports take place allowing moving the entire colony to the new site (Avargues-Weber and Monnin 2009). In *A. senilis*, the fact that the probability of colony relocation was higher when it had already occurred the week before suggests that the ants may successively try several nest locations until the best site is adopted for the next three to five weeks. After the ants have abandoned their nest, the whole structure of galleries and chambers most likely remains intact for a while which allows their further re-utilization by the same or other ants. Hence, the first suitable area discovered by a colony may, just by chance, be the one they had abandoned a few months earlier. In some occasions, a colony may also use a nest that was previously occupied by one of its neighbours (Boulay unpubl.). By doing so, the ants reduce the cost of excavating a new nest each time they have to relocate. This could explain the random pattern of colony relocation as well as the circular trajectories shown by our simulations.

Genetic structure and population viscosity

Our results are inline with a recently published microsatellite-based study (Chéron et al. 2009) which provided evidence that the socio-genetic organization of *A. senilis* colonies conforms to that of a monogynous–monoandrous system. Likewise, a strong genetic structuring within and among sampling sites was observed in our study. Considering the high mutation rate and bi-paternal inheritance mode of microsatellites, such a genetic structure has probably been stable for at least a few hundred generations. This suggests that, although gene flow between distant colonies may occasionally occur (most likely by flying males), such events are rare and not of sufficient magnitude to homogenize gene pools between interbreeding colonies. Such a pronounced genetic structure has also been observed in other fissioning ant species like *Diacamma cyaneiventre*, *Nothomyrmecia macrops* and *Cataglyphis cursor*, where divergence values (F_{ST} or analogous) between populations range from 3 to 15% (Doums et al. 2002, Sanetra and Crozier 2003, Clémencet et al. 2005). Interestingly, in these previous studies as well as in ours, there was a general lack of inbreeding and a relatively high genetic variability. In the case of *A. senilis*, this could be due to male-biased dispersal and/or some, perhaps yet unobserved kin-recognition mechanism that prevents inbreeding among nearby colonies.

On a very small scale (< 5 m), the spatial autocorrelation analysis showed relatedness values higher than expected by chance between any two random colonies within that distance (Fig. 5). This suggests that nearby colonies most likely share a common ancestor and so derived from relatively recent fission event(s). In contrast to species with independent colony foundation, colony fission drastically limits the distance at which effective dispersal is achieved. Available data in other ant species have found contrasting results when estimating population viscosity across a range of geographic distances depending on the genetic marker used (Seppä and Pamilo 1995, Chapuisat and Crozier 2001, Tsutsui and Case 2001, Berghoff et al. 2008). Some of these previous studies have evaluated the relationship between genetic similarities and geographic distance using both mitochondrial and nuclear markers. All results agree in a stronger genetic differentiation (orders of magnitude) at the mitochondrial level relative to nuclear loci, even within a few meters distance. This can be explained partly because the effective population size of the mitochondrial genome is only one quarter that of nuclear, and because dispersal is male-biased in most fissioning ants. In the present study we measured female dispersal directly (mark–recapture) and infer male dispersal indirectly by microsatellite markers. Our results indicate that males are mainly the dispersing sex and that they disperse over greater distances than fission events and colony relocations (female dispersal). Nonetheless, neither male nor female dispersal seem to prevent population viscosity within the short-distance range. This appears to be a general trend already well recognized in polygynous species that reproduce by colony fission (Chapuisat et al. 1997, Giraud et al. 2000, Tsutsui and Case 2001). However, recent genetic evidence suggests that population viscosity at micro-geographical scale can also be a common phenomenon in monogynous fissioning species (Doums et al. 2002, Sanetra and Crozier 2003). Thus, other traits such as male mating success and queen philopatric behaviour warrant further investigation.

Conclusion

Our results highlight the conceptual distinction between animal movement and effective dispersal (or gene flow). In species with dispersal polymorphism, effective dispersal is usually achieved by a fraction of the population with specialized phenotypes (including behaviour) moving through a landscape until settlement habitat is encountered and colonization (i.e. reproduction) takes place. In other species like fission-performing ants, dispersal occurs at the colony level and is limited by specific constrains such as the participation of apterous workers in fission. In this case, routine movements could acquire a particular importance to promote gene flow. However, our results suggest that the consequence of routine movements on population genetic structure will greatly depend on the orientation of successive displacements. Hence, in *A. senilis*, the lack of a constant directionality of successive relocations does not seem to outweigh the effects of limited dispersal as evidenced by high population viscosity within short distances. Occasional gene flow via male dispersal between nearby colonies appears to prevent inbreeding, but it is not of sufficient magnitude to completely cancel out population viscosity. The possibility of other cryptic inbreeding-avoidance mechanisms cannot be ruled out.

Overall, fission creates a neighbourhood area of genetically related colonies, which is maintained in spite of frequent relocations. A consequence of this could be the relatively low level of aggressiveness among neighbour colonies observed in this species, minimizing the effects of local-resource competition (Ichinose et al. 2009). Further studies on a variety of organisms, including other fission-performing ants, are necessary to address a possible evolutionary link between limited special-movement dispersal and enhanced routine movements as a regulatory process between population viscosity, inbreeding and local resource competition.

References

Adams, E. S. and Tschinkel, W. R. 2001. Mechanisms of population regulation in the fire ant *Solenopsis invicta*: an experimental study. – J. Anim. Ecol. 70: 355–369.

Amor, F. et al. 2011. The evolution of worker-queen polymorphism in *Cataglyphis* ants: interplay between natural and kin selection. – Behav. Ecol. Sociobiol. 65: 1473–1482.

Avargues-Weber, A. and Monnin, T. 2009. Dynamics of colony emigration in the ant *Aphaenogaster senilis*. – Insectes Soc. 56: 177–183.

Belkhir, K. et al. 1997. Genetix v.4.01 logiciel sous Windows TM pour la génétique des populations. – Lab. Génome, Populations, Interactions CNRS UPR 9060, Montpellier France.

Benjamini, Y. and Hochberg, Y. 1995. Controling the false discovery rate: a practical and powerfull approach to multiple testing. – J. R. Stat. Soc. B 57: 289–300.

Berghoff, S. M. et al. 2008. Dispersal and population structure of a New World predator, the army ant *Eciton burchellii*. – J. Evol. Biol. 21: 1125–1132.

Bollinger, E. K. et al. 1993. Inbreeding avoidance increases dispersal movements of the meadow vole. – Ecology 74: 1153–1156.

Boulay, R. et al. 2007. Intraspecific competition in the ant *Camponotus cruentatus*: should we expect the 'dear enemy' effect? – Anim. Behav. 74: 985–993.

Boulay, R. et al. 2009. Brood development into sexual females depends on the presence of a queen but not on temperature in an ant dispersing by colony fission, *Aphaenogaster senilis*. – Ecol. Entomol. 34: 595–602.

Boulay, R. et al. 2010. Intraspecific competition affects population size and resource allocation in an ant dispersing by colony fission. – Ecology 91: 3312–3321.

Bourke, A. F. G. and Franks, N. R. 1995. Social evolution in ants. – Princeton Univ. Press.

Brown, M. J. F. 1999. Nest relocation and encounters between colonies of the seed-harvesting ant *Messor andrei*. – Insectes Soc. 46: 66–70.

Cerdá, X. and Retana, J. 1998. Interference interactions and nest usurpation between two subordinate ant species. – Oecologia 113: 577–583.

Chapuisat, M. and Crozier, R. 2001. Low relatedness among cooperatively breeding workers of the greenhead ant *Rhytidoponera metallica*. – J. Evol. Biol. 14: 564–573.

Chapuisat, M. et al. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. – Evolution 51: 475–482.

Chéron, B. et al. 2009. Queen replacement in the monogynous ant *Aphaenogaster senilis*: supernumerary queens as life insurance. – Anim. Behav. 78: 1317–1325.

Chéron, B. et al. 2011. Unequal resource allocation among colonies produced by fission in the ant *Cataglyphis cursor*. – Ecology 92: 1448–1458.

Clémencet, J. et al. 2005. Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. – Mol. Ecol. 14: 3735–3744.

Clobert, J. et al. 2001. Dispersal. – Oxford Univ. Press.

Dahbi, A. et al. 2008. Nest-moving by the polydomous ant *Cataglyphis iberica*. – J. Ethol. 26: 119–126.

Dornhaus, A. et al. 2004. Ants move to improve: colonies of *Leptothorax albipennis* emigrate whenever they find a superior nest site. – Anim. Behav. 67: 959–963.

Doums, C. et al. 2002. Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. – Mol. Ecol. 11: 2251–2264.

Droual, R. 1984. Anti-predator behaviour in the ant *Pheidole desertorum*: the importance of multiple nests. – Anim. Behav. 32: 1054–1058.

Fahrig, L. and Merriam, G. 1985. Habitat patch connectivity and population survival. – Ecology 66: 1762–1768.

Franks, N. R. et al. 2003. Speed versus accuracy in collective decision making. – Proc. R. Soc. Lond. B 270: 2457–2463.

Franks, N. R. et al. 2007. Reconnaissance and latent learning in ants. – Proc. R. Soc. Lond. B 274: 1505–1509.

Fuller, B. W. et al. 1984. Relocation of red imported fire ant colonies. – J. Agric. Entomol. 1: 185–190.

Galarza, J. A. et al. 2009. Development of single sequence repeat markers for the ant *Aphaenogaster senilis* and cross-species amplification in *A. iberica, A. gibbosa, A. subterranea* and *Messor maroccanus*. – Conserv. Genet. 10: 519–521.

Galarza, J. A. et al. 2009. The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. – Proc. Natl Acad. Sci. 106: 1473–1478.

Gibb, H. and Houchuli, D. F. 2003. Nest relocation in the golden spiny ant, *Polyrhachis ammon*:environmental cues and temporal castes. – Insectes Soc. 50: 323–329.

Giraud, T. et al. 2000. Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. – Mol. Ecol. 9: 1835–1841.

Gordon, D. M. 1992. Nest relocation in harvester ants. – Ann. Entomol. Soc. Am. 85: 44–47.

Gordon, D. M. and Kulig, A. 1988. The effect of neighbours on the mortality of harvester ant colonies. – J. Anim. Ecol. 97: 141–148.

Greenwood, P. J. and Harvey, P. H. 1982. The natal and breeding dispersal of birds. – Annu. Rev. Ecol. Syst. 12: 1–21.

Hamilton, W. D. and May, R. M. 1977. Dispersal in stable habitats. – Nature 269: 578–581.

Hansson, L. 1991. Dispersal and connectivity in metapopulations. – Biol. J. Linn. Soc. 42: 89–103.

Hawkes, C. 2009. Linking movement behaviour, dispersal and population processes: is individual variation a key? – J. Anim. Ecol. 78: 894–906.

Heller, N. E. and Gordon, D. M. 2006. Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). – Ecol. Entomol. 31: 499–510.

Helmkampf, M. et al. 2008. Population- and sociogenetic structure of the leaf-cutter ant *Atta colombica* (Formicidae, Myrmicinae). – Insectes Soc. 44: 434–442.

Hölldobler, B. and Wilson, E. O. 1990. The ants. – Harvard Univ. Press.

Ichinose, K. et al. 2009. Influence of queen and diet on nestmate recognition and cuticular hydrocarbon differentiation in a fission-dispersing ant, *Aphaenogaster senilis*. – Zool. Sci. 26: 681–685.

Johnson, C. G. 1969. Migration and dispersal of insects by flight. – Methuen.

Keeler, K. 1988. Colony survivorship in *Pogonomyrmex occidentalis*, western harvester ant, in western Nebraska. – SW Nat. 33: 480–482.

King, R. L. and Sallee, R. M. 1956. On the half-life of nests of *Formica obscuripes* Forel. – Proc. Iwoa Acad. Sci. 63: 721–723.

Kisdi, V. 2004. Conditional dispersal under kin competition: extension of the Hamilton-May model to brood size-dependent dispersal. – Theor. Popul. Biol. 66: 369–380.

LaMon, B. and Topoff, H. 1981. Avoiding predation by army ants: defensive behaviours of three ant species of the genus *Camponotus*. – Anim. Behav. 29: 1070–1081.

Lenoir, A. et al. 1988. Reproduction and dispersal of the ant *Cataglyphis cursor* (Hymenoptera Formicidae). – Psyche 95: 21–44.

Mabelis, A. A. 1979. Nest splitting by the red wood ant (*Formica polyctena* Foerster). – Neth. J. Zool. 29: 109–125.

Maschwitz, U. and Hänel, H. 1985. The migrating herdsman *Dolichoderus (Diabolus) cuspidatus*: an ant with a novel mode of life. – Behav. Ecol. Sociobiol. 17: 171–184.

McGlynn, T. 2010. Serial monodomy in the gypsy ant, *Aphaenogaster araneoides*: does nest odor reduction influence colony relocation? – J. Insect Sci. 10: 195.

McGlynn, T. P. et al. 2002. Resource heterogeneity affects demography of the Costa Rican ant *Aphaenogaster araneoides*. – J. Trop. Ecol. 18: 231–244.

McGlynn, T. P. et al. 2004. Frequent nest relocation in the ant *Aphaenogaster araneoides*: resources, competition, and natural enemies. – Oikos 106: 611–621.

Molet, M. and Peeters, C. 2006. Evolution of wingless reproductives in ants: weakly specialized ergatoid queen instead of gamergates in *Platythyreaconradti*. – Insectes Soc. 53: 177–182.

Molet, M. et al. 2008. Shift in colonial reproductive strategy associated with a tropical–temperate gradient in *Rhytidoponera* ants. – Am. Nat. 172: 75–87.

Pamilo, P. 1991. Evolution of colony characteristics in social insects. 1. Sex allocation. – Am. Nat. 137: 83–107.

Peakall, R. and Smouse, P. E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. – Mol. Ecol. Notes 6: 288–295.

Peakall, R. et al. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. – Evolution 57: 1182–1195.

Peeters, C. and Ito, F. 2001. Colony dispersal and the evolution of queen morphology in social Hymenoptera. – Annu. Rev. Entomol. 46: 601–630.

Queller, D. C. and Goodnight, K. F. 1989. Estimating relatedness using genetic markers. – Evolution 43: 258–275.

Raymond, M. and Rousset, F. 1995. GENEPOP (ver. 3.4) population genetics software for exact tests and ecumenicism. – J. Hered. 86: 248–249.

Ronce, O. and Promislow, D. 2010. Kin competition, natal dispersal and the moulding of senescence by natural selection. – Proc. R. Soc. Lond. B 277: 3659–3667.

Sanetra, M. and Crozier, R. H. 2003. Patterns of population subdivision and gene flow in the ant *Nothomyrmecia macrops* reflected in microsatellite and mitochondrial DNA markers. – Mol. Ecol. 12: 2281–2295.

Schneider, S. et al. 2000. Arlequin ver. 2.0: a software for population genetics data analysis. – Genetics and Biometry Lab., Univ. of Geneva, Switzerland.

Seeley, T. D. 1997. Honey bee colonies are group level adaptive units. – Am. Nat. 150: 22–41.

Seppä, P. and Pamilo, P. 1995. Gene flow and population viscosity in Myrmica ants. – J. Hered. 74: 200–2009.

Smallwood, J. 1982. The effect of shade and competition of emigration rate in the ant *Aphaenogasterrudis*. – J. Ecol. 63: 124–134.

Tschinkel, W. R. 2006. The fire ants. – The Belknap Press of the Harvard Univ. Press.

Tsutsui, N. D. and Case, T. J. 2001. Population genetics and colony structure of the argentine ant (*Linepithema humile*) in its native and introduced ranges. – Evolution 55: 976–985.

Van Dyck, H. and Baguette, M. 2005. Dispersal behaviour in fragmented landscapes: routine or special movements? – Basic Appl. Ecol. 6: 535–545.

Verhoeven, K. et al. 2005. Implementing false discovery rate control: increasing your power. – Oikos 108: 643–647.

Wang, B. C. and Smith, T. B. 2002. Closing the seed dispersal loop. – Trends Ecol. Evol. 17: 379–385.

Waser, P. M. et al. 1986. When should animals tolerate inbreeding? – Am. Nat. 128: 529–537.

Weir, B. S. and Cockerham, C. C. 1984. Estimating F-statistics for the analysis of population structure. – Evolution 38: 1358–1370.

West, S. A. et al. 2001. Testing Hamilton's rule with competition between relatives. – Nature 409: 510–513.

Wiernasz, D. C. and Cole, B. J. 1995. Spatial distribution of *Pogonomyrmex occidentalis:* recruitment, mortality and overdispersion. – J. Anim. Ecol. 64: 519–527.

Wiernasz, D. C. and Cole, B. J. 2003. Queen size mediates queen survival and colony fitness in harvester ants. – Evolution 57: 2179–2183.

Wilson, D. et al. 1992. Can altruism evolve in purely viscous populations? – Evol. Ecol. 6: 331–341.

Appendix A1

Figure A1. *Aphaenogaster senilis* nest entrance ornamented with flower petals. Credit:
Alain Lenoir.

Table A1. Data from the 21 focal colonies used for mark-recapture and random-walk simulations.

Population	Colony ID	Date of release	Number of workers	Date last seen	Number of relocations	Average relocation distance (m)	Cumulated distance from first location (m)	Simulated cumulated distance from first location (m)
Beles	619	03/08/06	1620	09/13/06	9	3.9 ± 0.9	35.1	46.3 ± 14.7
	627	03/15/06	2243	05/17/06	4	3.4 ± 2.2	13.4	4.3±1.3
	628	03/08/06	454	05/31/06	4	5.8 ± 2.1	23.3	13.7 ± 2.6
	629	03/08/06	1569	11/01/06	7	4.0 ± 1.4	28.0	28.8 ± 9.0
	632	02/15/06	1203	10/18/06	8	4.3 ± 1.4	34.3	103.6 ± 40.7
	646	03/29/06	1108	06/14/06	4	4.3 ± 1.9	17.3	11.4 ± 2.4
	647	03/08/06	1786	06/07/06	5	3.5 ± 1.7	17.6	9.0 ± 2.5
Jaulon	601	03/08/06	1534	10/11/06	10	2.1 ± 0.9	21.3	23.6 ± 8.2
	602	02/08/06	1733	10/18/06	6	4.1 ± 1.6	24.5	48.2 ± 12.0
	604	02/08/06	1835	05/24/06	4	1.4 ± 0.4	1.9	8.1 ± 2.1
	605	02/08/06	1556	10/18/06	7	1.6 ± 0.3	11.3	19.4 ± 6.0
	606	05/03/06	1217	11/08/06	6	0.8 ± 0.1	3.8	6.2 ± 1.8
	608	04/05/06	646	11/08/06	7	2.0 ± 0.7	14.2	28.2 ± 6.3
	616	05/03/06	1836	10/04/06	3	2.6 ± 0.6	7.8	11.5 ± 3.8
	617	02/08/06	1203	10/11/06	10	1.1 ± 0.2	11.4	27.1 ± 8.7
Algaida	630	05/17/06	1192	06/21/06	3	4.7 ± 2.7	14.1	4.4 ± 1.2
	639	04/05/06	1628	06/07/06	4	4.2 ± 1.9	16.9	9.4 ± 3.0
	640	03/08/06	1467	10/25/06	8	5.1 ± 1.3	40.8	69.9 ± 25.6
	643	05/24/06	2132	09/27/06	7	6.1 ± 1.4	33.4	68.6 ± 20.2
	644	05/24/06	1055	08/16/06	6	4.6 ± 1.4	27.5	28.5 ± 8.7
	645	05/24/06	1502	08/16/06	7	3.9 ± 1.1	27.3	30.5 ± 9.6

Table A2. Spatial autocorrelation analyses for each population.

Pop *Comedero*

n	1	4	3	4	1	1	2	1	2	1	1	2	5	0	6	3	3	1	2	0
Distance Class (m)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
r	0.10	-2.87	-2.48	0.10	-3.28	0.43	-0.22	0.10	0.10	0.10	0.10	-5.42	0.62	0.00	1.71	1.32	1.64	0.46	0.10	0.00
U	2.25	1.50	2.63	3.67	1.98	2.66	2.58	2.25	2.62	2.66	1.98	2.74	1.16	0.00	2.59	2.19	1.53	1.72	1.68	0.00
L	-2.5	-2.26	-3.00	-3.57	-2.90	-1.69	-2.46	-2.90	-2.73	-3.28	-3.28	-4.48	-2.97	0.00	-3.05	-2.12	-2.56	-1.95	-2.08	0.00
p(r-rand >= r-data)	0.04	0.99	0.96	0.66	1.00	0.00	0.84	0.80	0.79	0.80	0.78	0.99	0.14	0.00	0.07	0.09	0.02	0.20	0.65	0.00

Pop *Visita*

n	5	14	13	8	0	0	7	3	6	8	2	1	3	3	5	6	2	2	2	2
Distance Class (m)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
r	2.04	1.09	-0.18	-4.81	0.00	0.00	-0.02	0.69	-0.20	0.50	2.20	0.05	-2.59	-1.05	1.61	0.50	0.37	1.06	1.49	0.05
U	2.59	1.13	1.76	1.06	0.00	0.00	1.25	2.28	2.05	1.42	2.30	2.50	1.98	2.03	1.34	2.00	2.13	2.11	1.58	2.56
L	-2.8	-1.37	-1.15	-2.22	0.00	0.00	-2.00	-1.51	-1.97	-1.90	-3.37	-2.46	-2.56	-3.82	-1.20	-2.03	-1.81	-2.23	-2.62	-1.96
p(r-rand >= r-data)	0.02	0.01	0.34	0.01	0.00	0.00	0.46	0.83	0.39	0.79	0.97	0.74	0.02	0.08	0.98	0.81	0.69	0.89	0.97	0.66

Pop *Beles*

n	6	21	21	5	4	3	10	13	10	17	10	25	25	19	18	4	8	6	9	7
Distance Class (m)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
r	0.11	0.04	-4.39	-1.14	0.04	-3.64	1.31	-3.12	-1.61	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	6.17	0.04
U	3.99	2.24	10.10	3.37	3.11	4.03	2.18	4.28	6.12	3.86	4.10	5.22	2.86	2.79	2.75	6.08	3.35	3.21	3.70	2.07
L	-3.5	-9.40	-7.30	-4.81	-5.13	-4.06	-2.81	-4.58	-4.32	-5.32	-5.62	-8.24	-2.90	-3.21	-12.3	-9.87	-3.27	-6.81	-3.63	-2.53
p(r-rand >= r-data)	0.06	0.86	0.95	0.92	0.85	0.06	0.08	0.96	0.94	0.92	0.87	0.88	0.86	0.79	0.88	0.91	0.89	0.88	0.02	0.87

N: number of pairwise comparisons per distance class. r: spatial autocorrelation and upper (U) and lower (L) 95% CI as determined by 999 random permutations.
p: probability of random achieving a *r* value grater or equal than the observed.

Table A3. AIC-based model selection. Details of backward model selection based on pairwise log likelihood test of concurrent models. The best model is indicated in bold. Preloc: probability of relocation; Dreloc: relocation distance; RelocPrev: having relocating the week befote or not; Wk: week of sampling; Sp: sampling site; Col: colony.

Probability of relocation

Model (R script)	AIC	Comparison	χ^2	DF	p
(1) Preloc~RelocPrev+Wk+(Wk|Sp/Col)	635.10				
(2) Preloc~RelocPrev+Wk+(1|Sp/Col)	629.38	1 vs 2	2.28	4	0.6842
(3) Preloc~RelocPrev+(1|Sp/Col)	627.66	2 vs 3	0.2806	1	0.5963
(4) Preloc~1+(1|Sp/Col)	640.00	3 vs 4	14.339	1	<0.001

Dispersal distance

Model (R script)	AIC	Comparison	χ^2	DF	p
(1) Dreloc~Wk+(Wk|Sp/Col)	345.59				
(2) Dreloc~Wk+(1|Sp/Col)	338.02	1 vs 2	0.4292	4	0.98
(3) Dreloc~1+(1|SpCol)	339.33	2 vs 3	3.2980	1	0.069