Nesting Behavior, Migration, and Dispersal of Neotropical Army Ants (Hymenoptera, Formicidae: Ecitoninae)

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Abstract

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Army ants, keystone predators in tropical forests, collectively hunt prey and are obligately nomadic. Despite a wide geographic and elevational range, the army ant *Eciton burchellii* is sensitive to environmental extremes and avoids entering deforested areas. Bivouac (nest) sites at high elevation were found to be more frequently located in sheltered locations than at low elevations (Chapter 1). High elevation bivouac sites provide abiotic buffering to *E. burchellii* colonies, though the effect declines at the highest elevations. The combination of forest clearing and high elevations may restrict army ant dispersal in Monteverde, Costa Rica. Restricted dispersal may lead to reduced gene flow, which threatens populations with inbreeding depression and local extinction. We compared individual-based pairwise relatedness estimates of reconstructed queen and male genotypes to resistance distances based on land cover and elevation (Chapter 2). Land cover (deforestation), but not high elevation, was

found to inhibit dispersal in army ants in Monteverde. Though significant spatial genetic structure was found in males but not queens, dispersal may be weakly male-biased. A temporal replication revealed no spatial pattern of relatedness over time (Chapter 3). This suggests that cumulative colony emigrations over the lifetime of the queen (before reproduction) may contribute to gene flow in this species. Thus forested habitat corridors should be maintained among fragments to allow for *E. burchellii* colony movements and subsequent gene dispersal. Army ants exhibit top-down effects on prey species and support a wide range of associates from mites to birds. Promoting army ant population persistence will maintain this biodiversity in tropical ecosystems.

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Chapter 1: Choice of nest site protects army ant colonies from environmental extremes in tropical montane forest

Abstract

Unlike most social insects, *Eciton burchellii* army ants can not thermoregulate through nest construction. Instead, army ants thermoregulate behaviorally by creating a living nest (*bivouac*), shifting its position and structure, and potentially through nest site selection. We hypothesized that bivouac site selection is critical to *E. burchellii* colony survival. We predicted elevation above sea level, with associated variation in local abiotic environments, would affect bivouac site selection by *E. burchellii* colonies. We also expected nest sites to buffer against ambient variation in abiotic conditions. We recorded bivouac site choice by *E. burchellii* colonies at sites ranging from lowland wet forests to montane forests and reviewed previously published data. We measured microclimatic variables associated with nest sites in high elevation montane forests: temperature, relative humidity, and light levels. Bivouac site selection varied with elevation: as elevation increased, fewer bivouac sites were exposed, more were underground, and fewer were elevated (in trees). High elevation bivouac sites moderated diurnal temperature variation and had higher relative humidity levels and lower light levels than ambient conditions. The buffering of ambient temperature and humidity decreased with elevation in montane forests, suggesting that abiotic extremes in bivouac sites at the highest elevations may contribute to the upper elevational range limits of *E. burchellii*.

Introduction

The nest of a social insect colony offers protection from temperature extremes and serves as an incubator for the brood (Seeley and Heinrich, 1981; Korb and Linsenmair, 1998; Starks and Gilley, 1999; Gardner et al., 2007; Penick and Tschinkel, 2008). Bees and wasps can actively thermoregulate through incubation, fanning, and water evaporation (Jones and Oldroyd, 2007). Relative to wasps and bees, ants have a limited capacity for metabolically regulating colony temperature (Seeley and Heinrich, 1981), and must rely on passive thermoregulation such as nest architecture and orientation (Jones and Oldroyd, 2007). For ant colonies that emigrate often, the choice of a nest site (*house-hunting*: Camazine et al., 1999) is perhaps the most important aspect of colony thermoregulation.

Army ants, keystone species in tropical forests (Franks, 1982a; Franks and Bossert, 1983; Gotwald, 1995; Kaspari and O'Donnell, 2003), do not construct nests. Instead, army ants use their bodies to form a living nest or *bivouac* without the manipulation of foreign materials (Schneirla et al., 1954). Most army ant species are hypogaeic, nesting and foraging underground, but the swarm-raiding army ant *Eciton burchellii* forages above ground (Hölldobler and Wilson, 1990; Gotwald, 1995). The bivouacs of *Eciton burchellii* colonies are often constructed in sheltered locations (bivouac sites); such as in hollow logs, mammal burrows, or between buttresses of large trees (Rettenmeyer, 1963). *E. burchelii* bivouacs may even be found hanging 20 m high in trees (Schneirla, 1971; S. O'D. pers. obs.).

Eciton burchellii army ant colonies forage *en masse* for prey in the leaf litter, may exhaust prey resources locally (Franks, 1982b; Otis et al., 1986), and emigrate frequently (Schneirla, 1971). During emigrations, the entire colony moves to a new bivouac site, often traversing linear distances of more than 100 m (Franks and Fletcher, 1983). This nomadism predictably occurs for 15 consecutive days, nearly half of the approximately 35 day *E. burchellii* brood cycle (Schneirla, 1971; Franks and Fletcher, 1983). Coinciding with the onset of this nomadic phase, tens of thousands of eggs have hatched into larvae and the colony must migrate in order to meet increased energy demands (Schneirla, 1971;

Hölldobler and Wilson, 1990; Gotwald, 1995). Army ants must therefore select a new bivouac site for each emigration during the nomadic phase. When entering the remaining 20 days of the brood cycle, or statary phase, army ants typically select one bivouac site, and raid outward from this central location (Franks and Fletcher, 1983). Coinciding with the onset of this statary phase, the larvae spin cocoons into pupae. During the statary phase the queen is physogastric and lays tens of thousands of eggs (Schneirla, 1971, Hölldobler and Wilson, 1990; Gotwald, 1995). The bivouac in the statary phase is more frequently sheltered (i.e. entirely hidden within a hollow log, mammal burrow, etc.) than in the nomadic phase (Schneirla et al., 1954).

The bivouac affords army ants some protection from ambient conditions. Army ant colonies are able to shift the bivouac position to avoid direct sunlight or precipitation (Schneirla et al., 1954; Rettenmeyer, 1963; Schneirla, 1971), and can regulate brood temperatures within the bivouac by opening and closing air passages (Jackson, 1957; Franks, 1989). However, the choice of the bivouac site may protect the colony from extreme environmental conditions, for example during the local dry season in lowland Panama (Rettenmeyer, 1963; Schneirla, 1971). Other social insect colonies select nest sites based on a range of criteria including cavity volume, entrance size, and light intensity (Visscher, 2007). Colonies of *E. burchellii* probably undergo a similar decision-making process when selecting a new bivouac site, and the ants may include microclimatic factors in this decision.

Despite the broad geographic and elevational range of *E. burchellii* (Watkins, 1976; Gotwald, 1995; O'Donnell and Kumar, 2006), individual army ants can only survive in a narrow range of environmental conditions (temperature: Meisel, 2006; humidity: Schneirla et al., 1954). Protection from ambient extremes is critical to colony survival. Unfavorable microclimates have been implicated in the disappearance of *E. burchellii* colonies, and the biodiversity they support, from forest fragments (Meisel, 2006; Kumar and O'Donnell, 2009) and agroecosystems (Roberts et al., 2000). Although in lowland forests, high temperatures and low relative humidity levels can be deadly to *Eciton* brood and workers

(Rettenmeyer, 1963; Schneirla, 1971; Meisel, 2006), army ants in premontane moist forest and lower montane wet forests are living near the upper limit of their elevational range in Costa Rica (O'Donnell et al., in press) and potentially, near the lower limit of their thermal tolerance. Because of climatic differences that accompany elevation increases in the tropics (e.g. lower temperatures: Clark et al., 2000), we expected montane *E. burchellii* colonies to seek more sheltered sites for their bivouacs than in the lowlands. Bivouac sites should be located underground more frequently to provide greater insulation and thermal buffering, and elevated (in trees) less frequently to minimize exposure to the environment, as elevation increases. We also predicted that high elevation sheltered bivouac sites protect an army ant colony, and its fragile queen and brood, from environmental extremes. Therefore, within bivouac site temperatures should be moderated compared to outside temperatures (i.e. warmer than ambient at night, cooler during the day). Bivouac site relative humidity levels should be moderated as well (i.e. higher during the day when risk of desiccation would be highest). Light levels should also be lower within the bivouac site, as the shelter physically shields ambient conditions. Low light levels may be a cue indicating covered shelter to the ants. We also assessed whether the amount of abiotic buffering provided by bivouac sites varied with elevation in montane habitats.

Methods

Study sites and subject colonies

We collected data on *E. burchellii* bivouac sites in Neotropical montane forest in the Monteverde area, Costa Rica (10°18'N, 84°47'W). Local habitats include primary forest reserves and adjacent forest fragments (Kumar and O'Donnell, 2007). We made observations in forests between 1000 m asl and 1680 m asl on the Pacific and Atlantic slopes in the vicinity of Monteverde, from July 1 to August 31 2009 during the rainy season.

We located *E. burchellii* colonies by conducting trail walks and by opportunistically following swarm-attending birds. We walked trails during daylight hours between 8:00 AM and 6:30 PM at a speed of approximately 1 km/h while visually scanning the ground surface for crossing raid columns or swarms (O'Donnell and Kumar, 2006; O'Donnell et al., 2007; Vidal-Riggs and Chavez-Campos, 2008). When a swarm or column of *E. burchellii* was found, we followed the column of ants back to the bivouac site. The bivouac and bivouac site were then qualitatively described using previously published site categories and classification criteria. The bivouac, when visible, was judged to be exposed if an estimated 50% or more of the circumference was exposed to the environment (e.g. risk of direct precipitation), and judged to be sheltered if mainly or entirely out of sight within a cavity (Schneirla et al., 1954; Teles da Silva, 1977). The bivouac was subterranean if located underground (not subterranean if not located underground) and elevated if located above ground level (not elevated if located at ground level or below) (Schneirla et al., 1954; Teles da Silva, 1977). Latitude, longitude and elevation of the bivouac site were taken to the nearest 10 m with a handheld GPS unit. Weather and distance permitting, we monitored colonies after dark to determine probable emigration status, and if emigrating, we followed colony emigrations to obtain successive bivouac locations (n = 21 bivouac sites from 9 colonies). We determined the reproductive phase of the colony (nomadic or statary) by monitoring the emigration schedule, observing the state of the brood during emigrations, and noting the presence of pupal cases outside the bivouac site.

We compared these data with data on qualitative bivouac descriptions collected in the Monteverde area (December 2007 and July 2010; S. O'D.), in premontane moist forest in Chiriquí, Panama (8º49'N, 82º45'W; 1200-1800 m asl; February-June 1996 and 1997; Roberts et al., 2000; Dina Roberts pers. comm.), in lowland moist forest and premontane moist forest in Henri Pittier National Park, Venezuela (10º21'N, 67º41'W; 500-1400 m asl; August 2003, May-June 2004, and April-May 2005; D. J. C. K.), in varzea forest in Cocha Cashu, Manu, Peru (11º55'S, 71º18'W; 400 m asl; September-

December 1999, October 2000-Februrary 2001, March-April 2002, March-May 2003, May-June 2007, and June-July 2008; S. K. W.), in lowland dry forest in Santa Rosa, Costa Rica (10º50’N, 85º37’W; 200 m asl; October-November 2008 and July 2009; S. O’D.), in lowland moist forest on Barro Colorado Island, Panama (9º9’N, 79º51’W; 0-137 m asl; May-December 1932, May-December 1933, May-December 1936, May-December 1938, January-April 1946, January-April 1948, May-December 1949, January-April 1952; Schneirla et al., 1954), and in varzea forest in Belém, Brazil (1º26’S, 48º23’W; <10 m asl; April 1966-June 1968; Teles da Silva, 1977).

Temperature and humidity measurements

We measured temperature and humidity after the ants had abandoned a bivouac site, so the colony’s raiding and emigration activities would not interfere with instrument placement and recording. We recorded temperature (°C) and humidity (%RH) every 5 minutes for over 24 hours with paired data loggers (Lascar Electronics model EL-USB-2-LCD) (*n* = 14 bivouac sites from 5 colonies). One data logger was placed on the substrate inside the bivouac site at the former location of the bivouac, or as far into the site as possible when the bivouac had not been visible. We suspended a second data logger vertically outside the bivouac site, 0.5 m away from the main entrance, with the recording end in contact with the substrate. The instrument was suspended to protect the data logger from heavy rain run-off (Lascar Electronics, pers. comm.). A protective barrier was also set up over the outside instrument. This barrier consisted of either a 0.5 m by 0.3 m plastic sheet hung 0.5 m above the data logger, at an angle to deflect rain, or a funnel with a 15 cm diameter hung 5 cm above the recording end of the data logger. Field tests indicated both the plastic sheet and the funnel had no effect on temperature and relative humidity readings, and were effective at deflecting precipitation. We alternated the data loggers between the inside and outside positions with each successive bivouac placement. Throughout the duration of the study, the data loggers, when not in the field, were operated adjacently to ensure continued calibration.

Light intensity measurements

We measured light intensity with a light meter (Mastech Digital Luxmeter model LX1330B) at each bivouac site at three different locations: within the bivouac site, at the main entrance to the site, and 0.5 m away from the main entrance (*n* = 17 bivouac sites from 6 colonies). When possible, we took repeated light intensity measurement series at a bivouac site at different periods of the day: morning (9-11 AM), midday (11:30 AM - 12:30 PM), and afternoon (2-4 PM); and under different local weather conditions: overcast and sunny.

Each individual measurement in a series was taken by recording the peak light intensity reading during a 10 second time span. For each measurement series, seven peak measurements were obtained sequentially, every ten seconds for seventy seconds: one peak measurement within the bivouac site (inside), a peak measurement at the entrance of the bivouac site, four peak measurements at different locations 0.5 m away from the entrance of the bivouac site (outside), and a second peak measurement at the entrance of the bivouac site. We took the inside measurement at the location of the bivouac when it had been visible, and as far into the site as possible when the bivouac had not been visible. At two bivouac sites, inside measurements were unavailable because the width of the entrance to the bivouac site was smaller than the width of the light meter probe.

Statistical analyses

We used logistic regression to analyze the bivouac site category data. The binary bivouac site categories (exposed/sheltered, subterranean/not subterranean, and elevated/not elevated) were the response variables, and phase (statary/nomadic) and elevation were the predictor variables (SAS 9.2). In all analyses, we used exact elevations of each bivouac site when available; otherwise we used the average elevation for the field site. We treated each bivouac site as an independent data point. We did not have data on colony identification at all field sites, and thus were unable to estimate colony effects on bivouac site selection across elevations.

Temperature and relative humidity measurements for a continuous 24 h period, beginning at least 30 min after the instruments had been last handled, were ordered by time of day. We calculated the average temperature and relative humidity for inside bivouac sites and that of 0.5 m outside bivouac sites at each time point (n = 288 time points). We calculated the variation in temperature and relative humidity for each bivouac site as the difference between the maximum and minimum measurements within a 24 h period. The average variation in temperature and relative humidity inside bivouac sites was compared to the average variation 0.5 m outside with paired Student's *t* tests (Microsoft Excel 2007).

To account for variations in local canopy cover, we averaged the two peak light intensity measurements taken at the entrance to the bivouac site to yield one entrance estimate, and the four peak light intensity measurements taken at different locations 0.5 m away from the entrance to yield one outside estimate. Thus, 15 bivouac sites had three light intensity measures each: inside, entrance, and outside. Two bivouac sites had only entrance and outside measures as we could not insert the light meter probe into the site. Because the light intensity data were not normally distributed, we compared light intensity across locations with a Kruskal-Wallis ANOVA (SAS 9.2). We performed post-hoc Wilcoxon signed rank tests for pairwise comparisons (SPSS 15.0). We also compared interior light levels, and the inside-outside light level difference, between exposed and sheltered bivouac sites with Mann-Whitney U tests (SPSS 15.0).

Lastly, we tested whether phase of colony development (statary vs. nomadic) and elevation affected the differences of bivouac site abiotic conditions from ambient conditions. We ran general linear models (GLM) on the inside-outside temperature difference at four different discrete time points: midnight (00:00 h), 6 AM (06:00 h), noon (12:00 h), and 6 PM (18:00 h) (SAS 9.2). The general linear models consisted of colony identity as the first covariate to account for colony effects, phase as the second covariate, and elevation as the final covariate. The GLM analyses were repeated for the humidity difference at the four time points, and for the average inside-outside difference in light intensity. We regressed the temperature difference (at all four discrete time points), ambient daytime temperature (average temperature from 06:00 - 17:55), ambient nighttime temperature (average temperature from 18:00 - 05:55), and the standard deviation of ambient temperature for the 24 h sampling period, on elevation.

Results

Effects of phase and elevation on bivouac site choice

Bivouac site characteristics covaried with both elevation and phase. The proportion of exposed bivouacs was lower in the statary phase (Wald $X^2 = 48.1$, $P < 0.0001$), and decreased as elevation increased (Wald $X^2 = 13.9$, $P < 0.001$, Fig. 1). As elevation increased, the decrease in proportion of exposed bivouacs did not differ between phases (elevation*phase interaction: Wald $X^2 = 0.65$, $P = 0.42$, Fig. 1). The proportion of subterranean bivouacs was greater in the statary phase (Wald $X^2 = 7.33$, $P < 0.01$) and increased as elevation increased (Wald $X^2 = 4.38$, $P < 0.05$, Fig. 2). As elevation increased, nomadic phase colonies showed a greater increase in the proportion of subterranean bivouacs (elevation*phase interaction: Wald $X^2 = 10.6$, $P < 0.01$, Fig. 2). The proportion of elevated (hanging) bivouacs was greater in the statary phase (Wald $X^2 = 52.9$, $P < 0.0001$) and decreased as elevation increased (Wald $X^2 = 5.87$, $P < 0.05$, Fig. 3). As elevation increased, the decrease in the proportion of elevated bivouacs was greater in the statary phase than in the nomadic phase (elevation*phase interaction: Wald $X^2 = 11.1$, $P < 0.001$, Fig. 3).

Bivouac site buffering of temperature and humidity variation and light intensity

Conditions within bivouac sites used by *E. burchellii* in the Monteverde area differed from ambient conditions. Night-time temperatures within bivouac sites were 0.5°C warmer (from 6 PM until 7 AM, Fig. 4a). Day-time temperatures within bivouac sites averaged 0.9°C cooler than ambient conditions (from 8:30 AM until 2:30 PM, Fig. 4a). The 24 h variation in temperature inside bivouac sites was significantly less than the variation of ambient temperature ($t_{13} = 5.85$, $P < 0.0001$, Fig. 4b). Relative humidity levels averaged 2.5% higher within bivouac sites during a large portion of the day (from 9:30 AM to 2:45 PM, Fig. 5a). The 24 h variation in relative humidity inside bivouac sites was significantly less than the variation of ambient relative humidity ($t_{13} = 3.12$, $P < 0.01$, Fig. 5b).

Light intensity differed among the three sampling locations at bivouac sites (outside, entrance, and inside; Kruskal-Wallis ANOVA: $X^2_2 = 52.9$, $P < 0.0001$). Bivouac sites had lower light intensity levels than ambient conditions. Averaged across all times of day and weather conditions, light levels within bivouac sites were less than 2% of light levels at entrances (Wilcoxon signed-rank test: $z = -3.41$, $P < 0.001$) and less than 1% of light levels outside bivouac sites (Wilcoxon signed-rank test: $z = -3.41$, $P < 0.001$). Average light levels at entrances were significantly less than light levels outside bivouac sites (Wilcoxon signed-rank test: $z = -2.77$, $P < 0.01$). Neither interior light levels nor the inside-outside difference in light intensity differed between sheltered bivouac sites and exposed bivouac sites (Mann-Whitney U tests: $z = -0.17$, $P = 0.865$ and $z = -1.36$, $P = 0.174$, respectively), though our limited sample of exposed sites constrained our ability to compare between these bivouac site categories.

Elevational variation in abiotic buffering

After accounting for the effects of colony and phase, elevation was a significant predictor of the temperature difference between inside and outside bivouac sites at midnight (00:00 h) and at 6 AM (06:00 h) ($P < 0.01$ at midnight and at 6 AM, Table 1). Buffering of ambient temperatures decreased with elevation at all four time points (Fig. 6). Elevation was not a significant predictor of the temperature difference at noon (12:00 h) and 6 PM (18:00 h). Elevation did not predict the humidity difference at all four time points (Table 1). General linear models predicting the average difference in light intensity levels from inside the bivouac site to outside the site were also not significant. Daytime and nighttime average temperatures declined with elevation ($R^2 = 0.61$, $P < 0.01$; and $R^2 = 0.49$, $P < 0.01$; respectively). The temperature variability over the full twenty-four hour cycle also decreased with elevation, but this trend was not significant ($R^2 = 0.09$, $P = 0.30$).

Discussion

Bivouac site choice

As elevation increased, *E. burchellii* colonies sought sheltered sites more frequently. Underground bivouac locations were used more frequently and elevated bivouac locations were used less frequently by army ant colonies with increasing elevation. Lower air temperatures due to adiabatic cooling at high elevations (Clark et al., 2000) may force *E. burchellii* colonies to bivouac in sheltered, subterranean, and non-elevated sites more often than colonies at low elevations. In general, ant nesting patterns change with latitude and climate (Seeley and Heinrich, 1981). In leaf-cutting ants of the genus *Acromyrmex* in lowland South America, as soil temperature increases, more species inhabit more deeply subterranean nests (Bollazzi et al., 2008). Soil saturation may also affect bivouac site selection. No subterranean bivouacs were observed in seasonally flooded varzea forest in Peru or Brazil (*n* = 400). Other factors at high elevations that could account for the observed changes in bivouac site selection behavior include an increased risk of physical disturbance (e.g. tree falls) and other climatic differences such as greater wind velocities or more extreme precipitation patterns. Though the role of predators and parasites on bivouac site selection is not known, one of the authors (S. K. W.) once observed an *E. burchellii* colony halt foraging and emigrate to a new bivouac site in response to a Giant Anteater (*Myrmecophaga* *tridactyla*) attack on the colony's foraging trails in lowland forest in Peru. This colony abandoned its exposed, terrestrial site for an exposed, elevated site 4 m high in a tree (Willson, 2003).

The bivouac site selection differences we documented could be due to plastic behavioral responses to local abiotic conditions, evolved (genetic) population differences, or a combination of the two. The wide elevational and latitudinal ranges of *E. burchellii*, combined with low queen dispersal (Berghoff et al., 2008; Jaffe et al., 2009) and the existence of morphologically distinct allopatric subspecies (Watkins, 1976), raise the possibility of local genetic adaptation to the abiotic environment. However, genetic adaptation may be limited by gene flow across elevations via dispersal of winged males. Though we do not have sufficient data to examine differences in bivouac site selection among *E. burchellii* subspecies, we know of no data on any other behavioral or physiological differences among subspecies of *E. burchellii*. More likely, colonies of *E. burchellii* have some behavioral plasticity in selecting bivouac sites, as evidenced by bivouac site selection varying with phase and season in lowland Panama (Schneirla et al., 1954) and in lowland Brazil (Teles da Silva, 1977), and differing during colony reproduction (Kronauer et al., 2007).

Abiotic buffering by bivouac sites

Although the bivouac itself is functionally important for colony thermoregulation in army ants (Franks, 1989), the choice of bivouac site may be crucial as well. Bivouac sites in Monteverde protected army ant colonies from environmental extremes. Diurnal temperature and relative humidity variation were moderated and light levels were lower within bivouac sites. Because temperature and humidity have a strong influence on individual survival (Schneirla et al., 1954; Meisel, 2006), these factors likely affect the process of bivouac site selection in this species. Light intensity has no effect on survival, though it is linked to individual and colony activity (Schneirla, 1971). Light levels therefore may serve as a cue indicating the presence of a sheltered area, and potentially a favorable microclimate for the near future.

Carl Rettenmeyer reported that the closely related *E. hamatum* will readily shift its clusters in response to heat and light in the laboratory, and will frequently shift bivouacs deeper into hollow logs in response to decreasing daytime humidity levels in the field (Rettenmeyer, 1963). Colonies of *E. burchellii* mostly emigrate at night (Schneirla, 1971; O'Donnell et al., 2009). A possible explanation for nocturnal emigrations may be reduced abiotic stresses on army ant brood (O'Donnell et al., 2009). *Eciton* emigrations are typically completed by midnight (Schneirla, 1971), thus army ant colonies in Monteverde would be able to take advantage of favorable microclimates at a new nomadic bivouac site during the later hours of the night and into the next day. Bivouac sites chosen by statary colonies are typically occupied for nearly three weeks (Schneirla, 1971; Franks and Fletcher, 1983), and the abiotic properties of these longer term sites may be especially important to colony fitness. However, limited queen mobility (due to physogastry) and/or different thermoregulatory requirements of different brood developmental stages may contribute to phase differences in bivouac site selection. Although emigrations are carried out nocturnally, *E. burchellii* colonies begin selecting potential bivouac sites during the late afternoon (Schneirla, 1971). Daytime selection of bivouac sites would enable colonies to more accurately predict abiotic buffering capabilities of potential bivouac sites. The relative values of physical protection, microclimate, and other nest site functions to the bivouac site selection process remain to be investigated.

Abiotic stressors, such as low temperature, may contribute to the upper elevational limits of *E. burchellii* in montane forests. We found that buffering of ambient temperatures in bivouac sites was reduced at higher elevations throughout the day and night. Furthermore, daytime and nighttime ambient temperatures decreased with elevation. These patterns suggest that bivouac sites provide weaker sheltering for army ant colonies at the elevations where the ants are most likely to be exposed to limiting low temperatures.

Conservation implications

The availability of *Eciton* bivouac sites could be important for protecting local biodiversity. The army ant *E. burchellii* exerts top-down effects on leaf litter communities, increasing local prey species diversity (Franks, 1982b; Franks and Bossert, 1983; Gotwald, 1995) and applying selection pressure on prey life history patterns (Gotwald, 1995; Kaspari and O'Donnell, 2003; Longino, 2005; McGlynn, 2007). Colonies of *E. burchellii* also support a wide array of associates, from microscopic mites to birds (Schneirla, 1971; Willis and Oniki, 1978; Gotwald, 1995; Kumar and O'Donnell, 2007). Our data suggest that sheltered bivouac sites are important to high-elevation *E. burchellii* populations. The lack of suitable bivouac sites may be one reason why colonies of *E. burchellii* disappear from agroecosystems, such as sun coffee plantations (Roberts et al., 2000). Therefore, leaving fallen logs in place to allow for *E. burchellii* inhabitance is a potentially important stewardship practice for supporting biodiversity in Neotropical montane forests. Furthermore, data on variation in bivouac site selection over large elevation ranges (0-1600 m) could provide a baseline for detecting effects of climate change on army ant ecology, as has been suggested for raiding behavior (O'Donnell and Kumar, 2006; Kumar and O'Donnell, 2007).

Conclusions

Although nest architecture and orientation are important for thermoregulation in many social insect colonies, nest site selection also contributes to colony thermoregulation (Seeley and Heinrich, 1981; Jones and Oldroyd, 2007). Social insect colonies are forced to find new nest sites for a variety of reasons, such as when the old nest is destroyed (Pratt et al., 2002; Franks et al., 2005), to avoid predation (McGlynn et al., 2004; Longino, 2005; McGlynn, 2007), during reproduction through colony fission (Seeley and Buhrman, 1999), in response to extreme weather events (Dejean et al., 2010), and even to thermoregulate (Seeley and Heinrich, 1981; Jones and Oldroyd, 2007). House-hunting, the process by which a social insect colony selects a new nest site, has been studied primarily in two genera: honeybees

(*Apis*) and rock ants (*Temnothorax*) (reviewed in Visscher, 2007). In those genera, individual scouts evaluate a range of criteria, such as cavity size, entrance size, light intensity, and even the presence of dead conspecifics (Franks et al., 2005), and recruit nestmates to the new nest site based on a weighted assessment of these multiple criteria (Visscher, 2007). The relative role of microclimate in nest site selection has not been systematically tested, but observational evidence suggests both single queens and mature colonies seek out locations with specific temperature and humidity before building a nest (Seeley and Heinrich, 1981; Jones and Oldroyd, 2007). Future work manipulating environmental conditions during the nest site selection process will elucidate the relationship between thermoregulation and house-hunting in social insect colonies.

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Tables

Table 1 General linear models predicting temperature difference and humidity difference with colony, phase, and elevation, at four different time points (midnight, 6 AM, noon, 6 PM).

DV	Time	full model	R^2	colony	phase	elevation
temp diff	00:00 h	**$F_{6,7} = 3.47$, $P = 0.064$**	0.749	$F_{4,7} = 1.31$, $P = 0.354$	$F_{1,7} = 0.22$, $P = 0.657$	**$F_{1,7} = 15.4$, $P < 0.01$**
temp diff	06:00 h	**$F_{6,7} = 3.71$, $P = 0.055$**	0.761	$F_{4,7} = 1.33$, $P = 0.349$	$F_{1,7} = 0.40$, $P = 0.548$	**$F_{1,7} = 16.6$, $P < 0.01$**
temp diff	12:00 h	$F_{6,7} = 0.81$, $P = 0.593$	0.410	$F_{4,7} = 0.45$, $P = 0.772$	$F_{1,7} = 1.22$, $P = 0.306$	$F_{1,7} = 1.85$, $P = 0.216$
temp diff	18:00 h	$F_{6,7} = 1.75$, $P = 0.239$	0.601	$F_{4,7} = 2.55$, $P = 0.133$	$F_{1,7} = 0.00$, $P = 1.00$	$F_{1,7} = 0.34$, $P = 0.579$
humid diff	00:00 h	$F_{6,7} = 0.62$, $P = 0.712$	0.347	$F_{4,7} = 0.13$, $P = 0.967$	$F_{1,7} = 2.76$, $P = 0.141$	$F_{1,7} = 0.44$, $P = 0.531$
humid diff	06:00 h	$F_{6,7} = 0.37$, $P = 0.875$	0.242	$F_{4,7} = 0.43$, $P = 0.786$	$F_{1,7} = 0.49$, $P = 0.507$	$F_{1,7} = 0.05$, $P = 0.829$
humid diff	12:00 h	$F_{6,7} = 0.49$, $P = 0.797$	0.297	$F_{4,7} = 0.53$, $P = 0.718$	$F_{1,7} = 0.82$, $P = 0.394$	$F_{1,7} = 0.00$, $P = 0.977$
humid diff	18:00 h	$F_{6,7} = 0.34$, $P = 0.893$	0.228	$F_{4,7} = 0.23$, $P = 0.913$	$F_{1,7} = 0.89$, $P = 0.377$	$F_{1,7} = 0.26$, $P = 0.626$

Figures

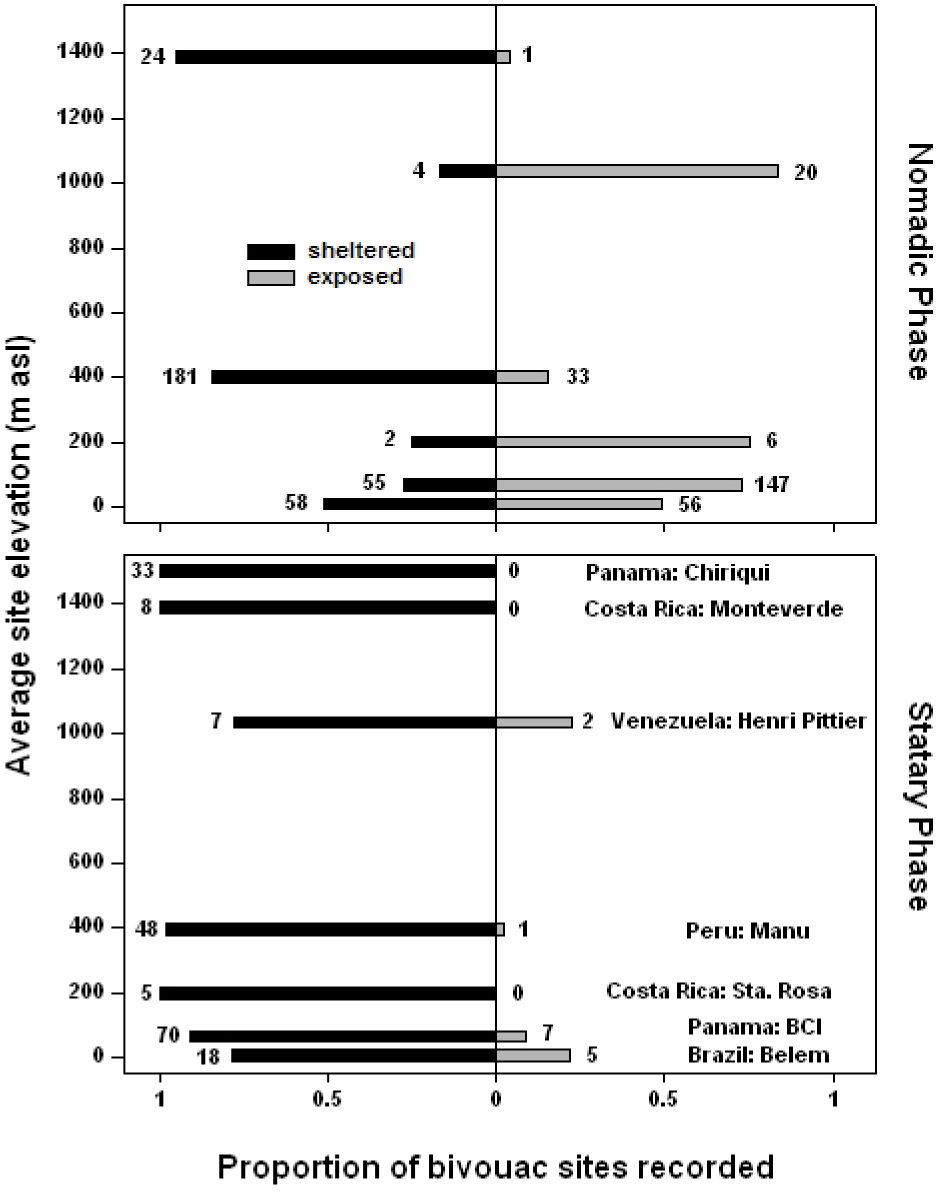

Fig. 1 Bar chart showing the frequency of *Eciton burchellii* exposed versus sheltered bivouac sites, at each site sampled (average elevation), by phase. Numbers to the left and to the right of bars denote sample sizes. No nomadic phase data were available for Chiriquí, Panama.

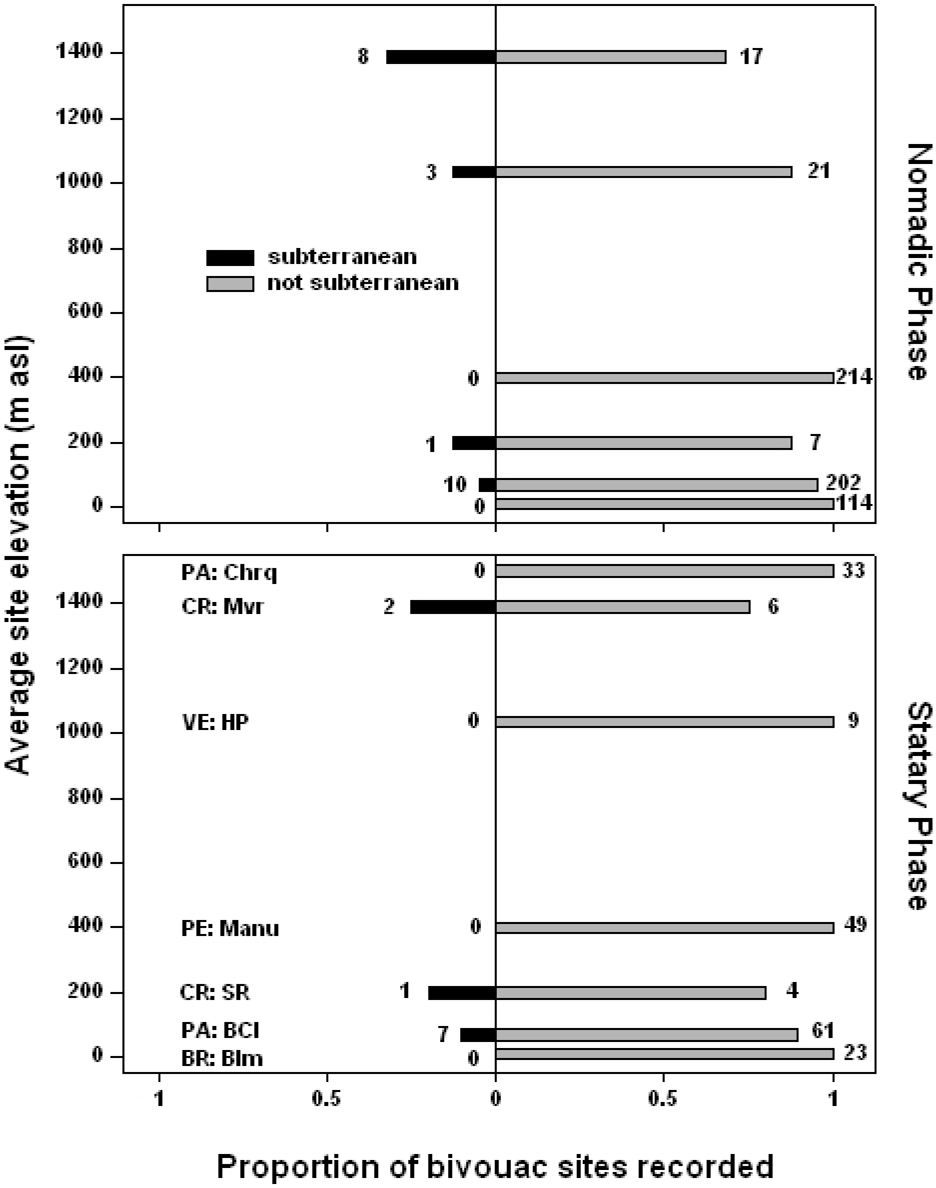

Fig. 2 Bar chart showing the frequency of *Eciton burchellii* subterranean bivouac sites, at each site sampled (average elevation), by phase. Numbers to the left and to the right of bars denote sample sizes. No nomadic phase data were available for Chiriquí, Panama.

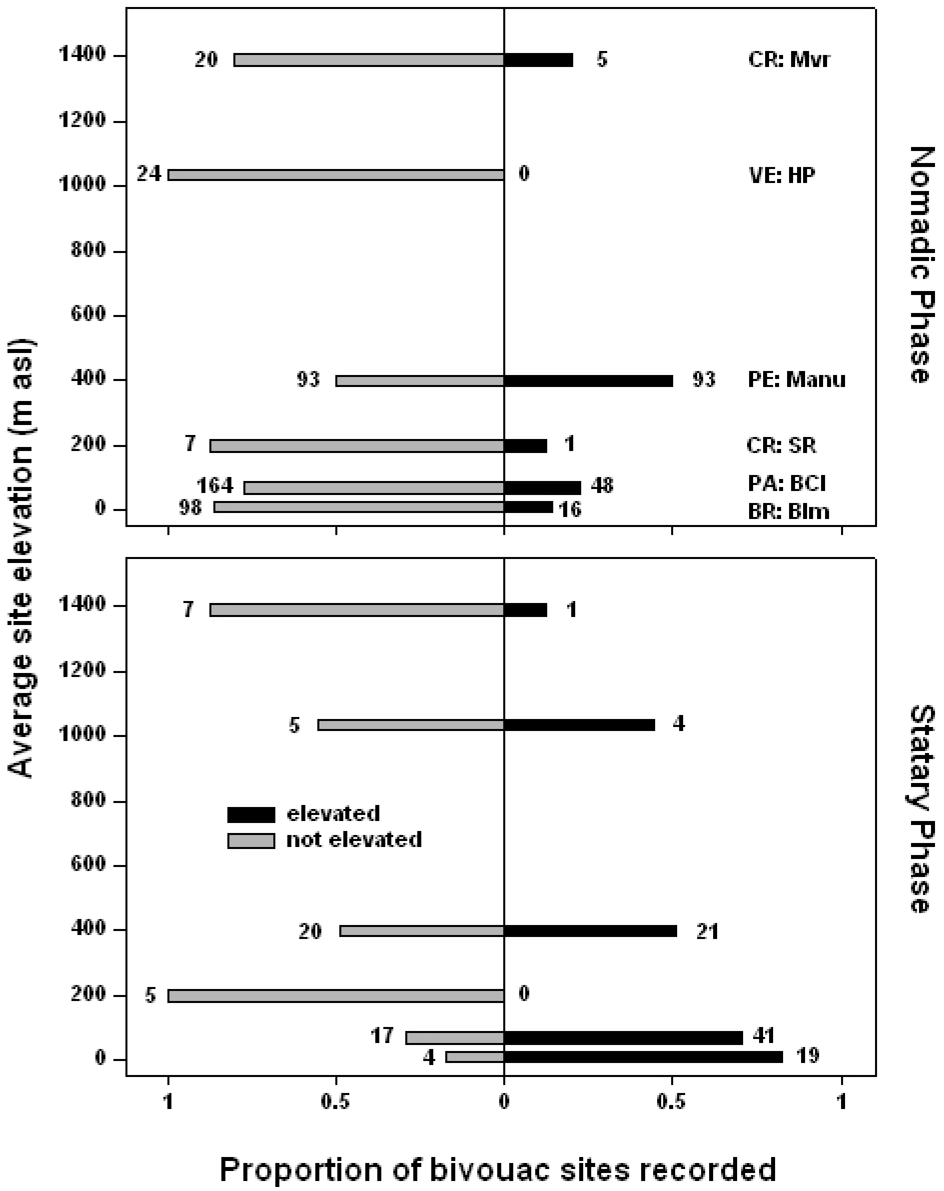

Fig. 3 Bar chart showing the frequency of *Eciton burchellii* elevated bivouac sites, at each site sampled (average elevation), by phase. Numbers to the left and to the right of bars denote sample sizes. No data were available for Chiriquí, Panama.

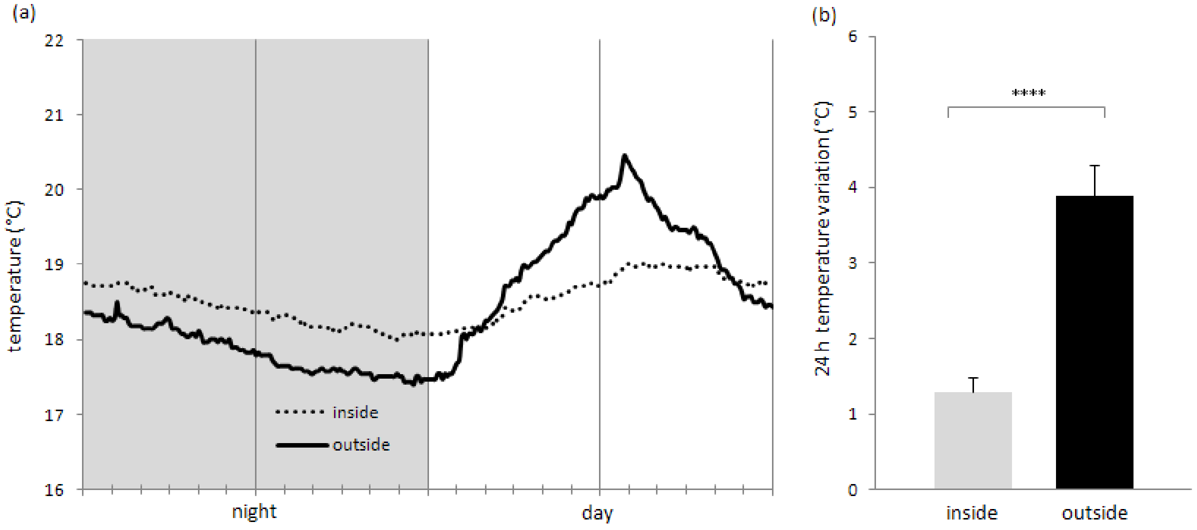

Fig. 4 (a) Line graph showing the average temperature inside the bivouac site (dotted line) and ambient conditions (solid line) over a 24 h period. Vertical lines represent the four time points at which general linear models (GLM) were run predicting the temperature difference (midnight, 6 AM, noon, and 6 PM). (b) Bar graph showing average 24 h temperature variation for inside the bivouac site (grey) and ambient conditions (black). Error bars represent one standard error from the mean.

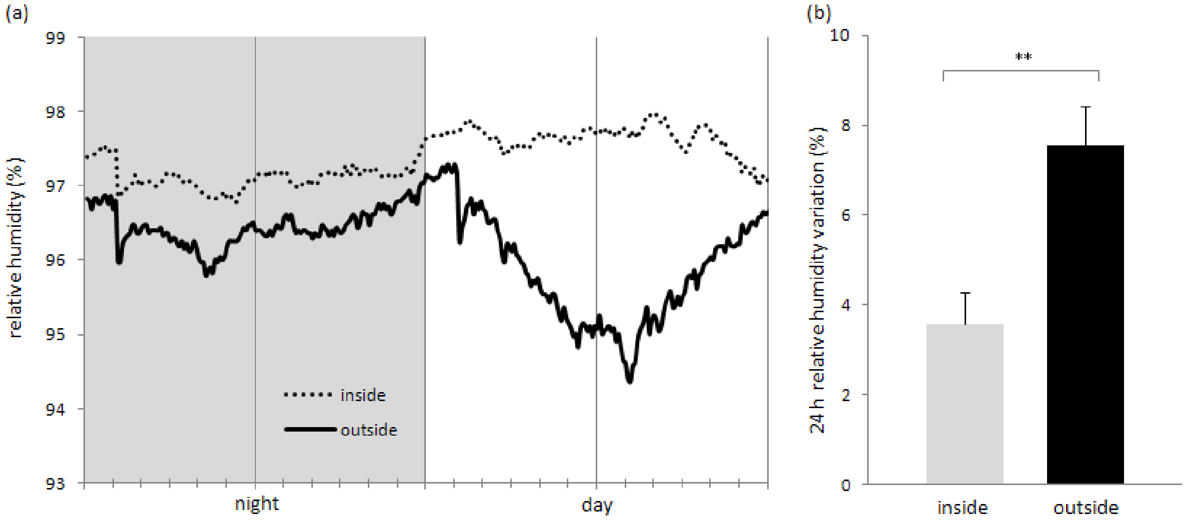

Fig. 5 (a) Line graph showing the average relative humidity inside the bivouac site (dotted line) and ambient conditions (solid line) over a 24 h period. Vertical lines represent the four time points at which general linear models (GLM) were run predicting the relative humidity difference (midnight, 6 AM, noon, and 6 PM). (b) Bar graph showing average 24 h relative humidity variation for inside the bivouac site (grey) and ambient conditions (black). Error bars represent one standard error from the mean.

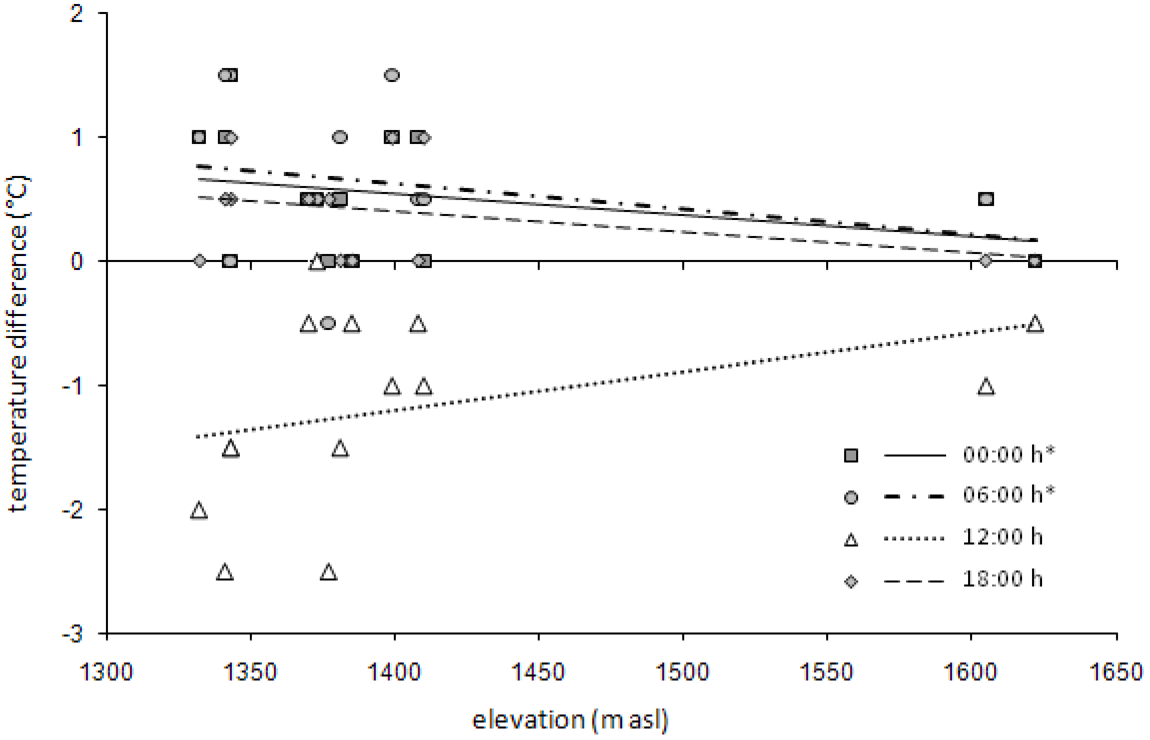

Fig. 6 Temperature differences by elevation in the Monteverde area. Asterisk (*) denotes the time points for which elevation is a significant predictor of temperature differences, after the effects of colony and phase are removed, as presented in the above models (Table 1).

Chapter 2: Genetic evidence for landscape effects on dispersal in the army ant *Eciton burchellii*

Abstract

Inhibited dispersal, leading to reduced gene flow, threatens populations with inbreeding depression and local extinction. Fragmentation may be especially detrimental to social insects (haplodiploids) because inhibited gene flow has important consequences for life histories, mating systems, and interactions within and among colonies. Army ants have winged males and permanently wingless queens; these traits imply male-biased dispersal. However, army ant colonies are obligately nomadic and have the potential to traverse landscapes. *Eciton burchellii*, the most regularly nomadic army ant, is a forest interior species: colony raiding activities are limited in the absence of forest cover. To examine whether nomadism and landscape (forest clearing and elevation) affect population genetic structure in a montane *E. burchellii* population we reconstructed queen and male genotypes from 25 colonies at seven polymorphic microsatellite loci. Pairwise genetic distances among individuals were compared to pairwise geographic and resistance distances using regressions with permutations, partial Mantel tests, and random forests analyses. Though there was no significant spatial genetic structuring in queens or males in montane forest, dispersal may be male-biased. We found significant isolation by landscape resistance for queens based on land cover (forest clearing), but not on elevation. Summed colony emigrations over the lifetime of the queen may contribute to gene flow in this species and forest clearing impedes these movements and subsequent gene dispersal. Further forest cover removal may increasingly inhibit *Eciton burchellii* colony dispersal. We recommend maintaining habitat connectivity in tropical forests to promote population persistence for this keystone species.

Introduction

Dispersal has profound evolutionary consequences for many levels of biological organization (Broquet & Petit 2009). In continuous populations, the interaction between dispersal and genetic drift may lead to isolation by distance (Slatkin & Maddison 1990; Hardy & Vekemans 1999) and the balance between dispersal and selection can generate adaptive evolution and speciation (Turelli *et al.* 2001). Dispersal restricted by habitat fragmentation can create inbreeding depression within subpopulations and even local extinction (Saccheri *et al.* 1998; Segelbacher *et al.* 2010). Habitat fragmentation can inhibit gene flow by isolating populations, increasing edge effects, and changing species biology (Fischer & Lindenmayer 2007). In tropical forest ecosystems, habitat fragmentation has altered species richness, abundances, and interactions; and affected ecosystem processes such as nutrient cycling (Bierregaard *et al.* 1992; Laurance *et al.* 2002).

Fragmentation may have significant effects on social insects. These species (Hymenoptera) are haplodiploid and generally have a lower effective population size and less molecular variation than diploids (Hedrick & Parker 1997). Gene flow in social insects may have important consequences for mating systems (e.g. number of breeders, reproductive skew, worker policing); life histories (e.g. mode of colony founding); and cooperation and competition within colonies, among colonies, and among populations (Pamilo *et al.* 1997; Ross 2001). Evolutionary processes are of particular interest in nomadic army ants (*Eciton burchellii*) because they are top predators and ecological keystones in tropical forests (Franks 1982; Franks & Bossert 1983; Kaspari *et al.* 2011); army ant colonies support over 300 species of animal associates (Rettenmeyer *et al.* 2011). Factors that affect army ant population structure and viability may cascade among these species (Koh *et al.* 2004). Despite regular obligate nomadism and a wide geographical range, *Eciton burchellii* army ants are sensitive to abiotic conditions (Schneirla *et al.* 1954; Meisel 2006) and their populations are vulnerable to local extinction in forest fragments (Partridge *et al.* 1996; Boswell *et al.* 1998; Meisel 2004) and agroecosystems (Roberts *et al.* 2000). *Eciton* *burchellii* avoids entering open areas in lowland rainforest (Meisel 2006), but increasingly forages in open areas at high elevations (Kumar & O'Donnell 2009). In addition, fragmentation in Costa Rica has been found to decrease swarm attendance and species richness of army ant-following birds (Kumar & O'Donnell 2007).

In undisturbed ant populations, dispersal is primarily determined by the mode of colony founding: independent founding by flying queens or dependent founding where queens are accompanied by flightless workers (Pamilo *et al.* 1997; Peeters & Ito 2001; Ross 2001). Dispersal for dependent foundresses is determined by the distance over which they can walk, and therefore is more limited than that of independent foundresses (Pamilo *et al.* 1997; Peeters & Ito 2001; Sundström *et al.* 2005). Male ants are winged in most species (Hölldobler & Wilson 1990); therefore male-biased dispersal is more strongly associated with dependent colony founding in ants (Seppä & Pamilo 1995; Pamilo *et al.* 1997; Sundström *et al.* 2005).

Eciton burchellii army ant colonies are monogynous and reproduce by fission: two giant wingless queens each walk away with half of the worker force (Schneirla 1971; Gotwald 1995; Kronauer 2009). Virgin queens mate immediately with multiple males and store this sperm for the rest of their lives (Kronauer *et al.* 2006; Kronauer & Boomsma 2007). In a stable population on Barro Colorado Island, Panama, fission occurs approximately every three years (Franks 1985; Kronauer 2009). Because army ants reproduce by colony fission but males are winged, dispersal may be male-biased. Previous genetic studies of army ants have detected high rates of polyandry (Kronauer *et al.* 2006) and evidence for male-biased dispersal (Berghoff *et al.* 2008; Pérez-Espona *et al.* 2012a). However, army ant colonies have a regular nomadic period tied to their five week reproductive cycle: nomadic for about two weeks while the larvae are growing (Schneirla 1971; Franks & Fletcher 1983). Within a nomadic phase, *Eciton burchellii* colonies roughly follow a single compass bearing (Franks & Fletcher 1983; Willson *et al.* 2011; but see Califano & Chaves-Campos 2011). Consecutive statary bivouacs (temporary nests separated by entire nomadic phases) are located a greater distance apart (*c.* 500 m) than what would be expected if the colonies simply performed a random walk throughout the nomadic phase (Franks & Fletcher 1983; Willson *et al.* 2011). In Chiapas, Mexico, male dispersal was estimated to be less than 1 km (Jaffé *et al.* 2009). Therefore regular colony emigrations have the potential to contribute to gene flow in nomadic army ants.

Settled by North American Quakers in the 1950s as primarily pasture for dairy production, substantial forest conservation began in the area of Monteverde, Costa Rica, in the 1970s, alongside a burgeoning ecotourism industry and continued residential development (Nadkarni & Wheelwright 2000). The Atlantic slope of Monteverde has experienced substantial reforestation since the 1980s, but much of the Pacific slope primary forest remains fragmented (Wheelwright 2000). Deforestation in lowland forest in Panama was found to inhibit *E. burchellii* dispersal (Pérez-Espona *et al.* 2012a). Fragmentation in the Monteverde region could likewise inhibit colony emigrations and decrease subsequent gene flow. Furthermore, *Eciton burchellii* colonies in Monteverde are likely living at the lower limit of their thermal tolerance (O'Donnell & Kumar 2006; O'Donnell *et al.* 2011; Soare *et al.* 2011). High elevations in the area may uniformly hinder colony emigrations and dispersal. Alternatively, colonies may be locally adapted to and may preferentially disperse within their natal elevation band; elevation bands are coarsely correlated with Holdridge life zones (Holdridge 1967) in the Monteverde area (Haber 2000). Thus, forest clearing on Monteverde slopes allows the examination of the effects of both habitat fragmentation and elevation on populations (e.g. Kumar & O'Donnell 2007, 2009). Army ant males disperse by flying, but colonies stay mainly in the forest interior (Meisel 2006; Kumar & O'Donnell 2009). Thus landscape variables may primarily affect queen dispersal by restricting colony emigrations. Males may cross landscape barriers such as the waters of the Panama Canal and major rivers (Berghoff *et al.* 2008; Pérez-Espona *et al.* 2012a), though deforestation has been found to inhibit (and mature forest promote) gene flow measured with nuclear markers (Pérez-Espona *et al.* 2012a).

In social insects, traditional population genetic statistics (e.g. Wright's fixation index, F_{ST}: Weir & Cockerham 1984 and analogues) have been used to measure nuclear DNA differentiation among subpopulations comprised of colonies and compared to subpopulation differentiation of mitochondrial DNA (mtDNA) to detect sex-biased dispersal (Doums *et al.* 2002; Clemencet *et al.* 2005; Berghoff *et al.* 2008; Foitzik *et al.* 2009). A recent analysis of sex-biased dispersal in army ants compared mtDNA differentiation to that of nuclear microsatellites among colonies (Pérez-Espona *et al.* 2012a). However, mtDNA has a relatively low mutation rate and may lack the spatial and temporal resolution to detect recent gene flow (Wang 2010, 2011a). Pairwise relatedness estimates based on nuclear DNA compared among *individuals* can detect fine scale spatial genetic structure (Loiselle *et al.* 1995; Rousset 2000; Hardy & Vekemans 1999; Vekemans & Hardy 2004; Nussey *et al.* 2005; Broquet & Petit 2009; Segelbacher *et al.* 2010; Ivens *et al.* 2012), including in ants (Seppä & Pamilo 1995; Doums *et al.* 2002; Sundström *et al.* 2003; Clemencet *et al.* 2005; Kronauer *et al.* 2010; Sanllorente *et al.* 2010; Suni & Gordon 2010). Individual pairwise relatedness approaches based on nuclear loci *compared across sexes* has detected sex-biased dispersal within single generations in a wide range of animals (Hazlitt *et al.* 2004; Hardy *et al.* 2008; Huck *et al.* 2008; Ortego *et al.* 2011; Messier *et al.* 2012; Colson *et al.* 2013). In simulations, the individual-based approach has detected weak spatial genetic structuring (Jones & Wang 2012) and sex-biased dispersal after a single generation (Banks & Peakall 2012). Thus we use this method to examine sex-biased dispersal in two populations of the army ant *Eciton burchellii*.

The specific aim of this study was to determine whether landscape variables (forest fragmentation and elevation) affect recent gene flow in army ants. To achieve this aim, we determined whether colony emigrations contributed to gene flow in army ants by comparing spatial genetic structure across castes (workers, queens, and males) sampled after dispersal. We then compared relatedness patterns among queens with competing isolation by landscape resistance models to examine the effects of forest clearing and elevation on colony dispersal. We used a landscape genetics approach and sampled at the level of individuals without identifying populations in advance, enabling detection of spatial genetic structure (e.g. isolation by distance patterns: Hazlitt *et al.* 2004; Hardy *et al.* 2008; Banks & Peakall 2012) and comparison of relative influence of landscape variables on contemporary gene flow (Manel *et al.* 2003; Segelbacher *et al.* 2010; Storfer *et al.* 2010; Short Bull *et al.* 2011).

Methods

Study site and samples

We sampled *Eciton burchellii* colonies in Neotropical montane forest around Monteverde, Costa Rica (10°18′ N, 84°48′ W) from 15 Jul 2006 to 16 Sep 2006. The Monteverde area lies in the Tilarán mountain range and straddles the continental divide (Nadkarni & Wheelwright 2000). The area consists of primary forest reserves, secondary forest, agricultural lands (e.g. pasture), residential development, and forest fragments (Nadkarni & Wheelwright 2000; Kumar & O'Donnell 2007). We located colonies through systematic trail walks and opportunistic encounters (Vidal-Riggs & Chaves-Campos 2008; Kumar & O'Donnell 2009; Soare *et al.* 2011), thus assuming a continuously distributed population and independence of observations (Storfer *et al.* 2007). We also attempted to maximize pairwise geographic and elevational distance between collections. Because patrilines contribute non-randomly to different worker castes in *E. burchellii* (Jaffé *et al.* 2007), we collected 13-30 worker ants of all castes from raiding columns directly into 95% EtOH. For all collections, we obtained latitude, longitude, and elevation to the nearest 10 m with a handheld GPS unit. 57 total collections were reduced to 25 for genetic analysis. We were reasonably certain these collections were from different colonies: subsets of the 25 colonies had been collected in the same area on the same day, and raid columns were followed back to the bivouac (nest) sites to ensure the collections were from different colonies. We sampled colonies on both sides of the continental divide (Pacific slope: 1000 – 1550 m asl, Atlantic slope: 1200 – 1400 m asl) over 4.6 km E-

W and 9.3 km N-S (an area of approximately 29 km^2, calculated as a polygon containing all sample points). All samples were subspecies *E. b. parvispinum* Forel (1899).

Genotyping

We extracted DNA from samples using a standard Chelex protocol (Walsh *et al.* 1991). Eight polymorphic loci were amplified for genotyping: *Eb04*, *Eb10*, *Eb14*, *Eb21*, *Eb24*, *Eb25*, *Eb42* and *Eb51* (Denny *et al.* 2004; Kronauer *et al.* 2006; Berghoff *et al.* 2008; Jaffé *et al.* 2009; Pérez-Espona *et al.* 2012a). One locus (*Eb14*) was dropped due to poor amplifications. Details of polymerase chain reaction (PCR) conditions for fragment amplification, including annealing temperatures for each locus, can be found in the Supporting information (Table S1). Fragment analyses were performed with ABI 3100xl and ABI 3730 sequencers (Applied Biosystems, Carlsbad, CA, USA) and scored in GeneMapper 4.0 software (Applied Biosystems). Over 11.5% of samples were successfully run on both sequencers, allowing us to cross-calibrate and standardize allele scores (DeWoody *et al.* 2006; Selkoe & Toonen 2006; Seeb *et al.* 2007). All allele scores were checked manually. A subset of samples was re-scored to estimate error (DeWoody *et al.* 2006; Selkoe & Toonen 2006; Guichoux *et al.* 2011); the error rate was 0.1%.

Genetic analysis

We reconstructed queen and male genotypes from all worker genotypes in the program COLONY 2.0 (Jones & Wang 2010). COLONY reconstructs queen and male genotypes simultaneously using a maximum likelihood technique, while updating allele frequencies based on previously defined relationships (i.e. nestmates), and thus provides a superior mode for reconstructing reproductive genotypes (Jones *et al.* 2010; Jones & Wang 2010). To test the effects of genotyping error and to confirm reliability, we repeated COLONY analyses with different error rates and random number seeds. We calculated overall and within locus inbreeding among queen genotypes (F_{Iq}) in SPAGeDi 1.3 (Hardy &

Vekemans 2002) and tested for linkage disequilibrium among all pairs of loci in queen genotypes using FSTAT 2.9.3.2 with 420 permutations (nominal $P = 0.0024$) (Goudet 2002). The effective mating frequency (Nielsen *et al.* 2003) of army ant queens in Monteverde, Costa Rica was compared with published data on queens from Barro Colorado Island, Panama (Kronauer *et al.* 2006); Henri Pittier National Park, Venezuela (Kronauer & Boomsma 2007); and Chiapas, Mexico (Jaffé *et al.* 2009) with a Kruskal-Wallis ANOVA in the stats package in R 2.14.1 (R Development Core Team 2011).

Power analysis

To test the accuracy of pairwise measures of genetic similarity based on the observed allele frequencies at the seven microsatellite loci, we simulated queen genotypes of known relationship levels in COANCESTRY 1.0 (Wang 2011b). We created 25 pairs of queen genotypes based on the population allele frequencies calculated by COLONY, with no inbreeding, in each of five relatedness levels: $r_{true} = 0.5$ (e.g. parent-offspring and full sibships), $r_{true} = 0.25$ (e.g. half-sibs and avuncular), $r_{true} = 0.125$ (e.g. first cousins), $r_{true} = 0.0313$ (e.g. second cousins) and $r_{true} = 0$ (unrelated). We then calculated the pairwise kinship coefficient (F_{ij}) and the pairwise relationship coefficient (r_{ij}) for each dyad in SPAGeDi. We compared true pairwise relatedness (r_{true}) with the relatedness estimates from SPAGeDi with linear regression and calculated differences among predicted relatedness levels with ANOVA in the stats package in R 2.14.1 (R Development Core Team 2011).

Comparison of dispersal distances for queens, males, and workers

Examining individual-based pairwise relatedness estimates based on nuclear loci across sexes can detect sex-biased dispersal (Hazlitt *et al.* 2004; Hardy *et al.* 2008; Banks & Peakall 2012). Although common in the social insect literature, the pairwise relationship coefficient (r_{ij}: Queller & Goodnight 1989) is asymmetrical when comparing individuals differing in ploidy or inbreeding levels; and thus the pairwise kinship coefficient (F_{ij}: Loiselle *et al.* 1995) allows direct comparison of dispersal abilities across sexes in haplodiploids within a single generation (Hardy *et al.* 2008; Sanllorente *et al.* 2010). Therefore, we used pairwise kinship (F_{ij}) in all regression analyses testing for sex-biased dispersal.

To examine sex-biased dispersal, we tested the relationship between pairwise kinship (F_{ij}: Loiselle *et al.* 1995) among queens, males, and workers and the natural logarithm of Euclidean distance between colonies using regression and jackknifing over loci in SPAGeDi 1.3 (Hardy & Vekemans 2002). Under sex-biased dispersal, the degree of spatial genetic structuring in workers should be intermediate between that of the dispersing sex and that of the more philopatric sex (Hardy *et al.* 2008). We obtained two-tailed *P* values by permuting locations of individuals 10000 times, and comparing the observed slope of the regression line to the mean permuted value, which is the equivalent of a Mantel test (Hardy & Vekemans 2002; Hardy *et al.* 2008). The level of significance obtained from such permutation tests (or Mantel tests as below) can be considered as a test of the significance of the observed data compared to the null hypothesis of no relationship (Manly 1997; Legendre & Legendre 1998). Because our sampling scheme was two-dimensional, pairwise kinship between individuals should scale with the natural logarithm of Euclidean distance (Rousset 1997; Hardy & Vekemans 1999; Vekemans & Hardy 2004; McRae 2006; Hardy *et al.* 2008), and thus the transformed distance was used in all analyses (hereafter "Euclidean distance"). Similarly, we also regressed pairwise kinship between individuals in SPAGeDi over the following preselected spatial extents: 0-7 km, 0-4 km, 0-2 km, 0-1 km, and 0-0.5 km. The proportion of individuals represented in each distance interval and the coefficient of variation (CV) of the number of times each individual is represented in each interval conformed to the suggestions of the software authors, with the exception of the maximum distance interval (7-9.3 km), which had a CV greater than one (Hardy & Vekemans 2002). Undersampling of individuals in the maximum interval could introduce bias into the computation of average pairwise kinship for that distance interval. Comparisons of individuals within the same colony (i.e. for males and workers) were not included in regression analyses (Hardy & Vekemans 2002).

We compared our data to a recent landscape genetics study of a population of *Eciton burchellii foreli* Mayr (1886) in Panama (Pérez-Espona *et al.* 2012a, 2012b). (See Pérez-Espona *et al.* 2012a for details on sample collection and genotyping.) Although six microsatellite loci were amplified in both populations, we could not combine the genetic data from Monteverde with that from San Lorenzo, Panama into a single analysis because markers were re-designed for the San Lorenzo population (S. Pérez-Espona *pers comm.*) and the general difficulties associated with standardizing allele scores (Seeb *et al.* 2007). To facilitate a direct comparison of sex-biased dispersal across populations, we regressed pairwise kinship (F_{ij}) for queens and males (genotypes obtained from 15 colonies: Pérez-Espona *et al.* 2012b) on the natural logarithm of Euclidean distance and restricted distances (0-9.3 km, 0-7 km, 0-4 km, 0-2 km, 0-1 km, and 0-0.5 km) in SPAGeDi 1.4 (Hardy & Vekemans 2002). The proportion of individuals represented in each distance interval and the coefficient of variation (CV) of the number of times each individual is represented in each interval did not comply with the suggestions of the software authors for the three smallest distance intervals. As above, undersampling of individuals within these intervals could introduce bias into the computation of average pairwise kinship for these distance intervals (Hardy & Vekemans 2002).

Spatial analysis and evaluating effects of landscape

To determine if land cover (forest clearing) and elevation impede colony migrations (queen dispersal), we followed a landscape genetics approach. Samples were treated as being derived from a continuous population and all analyses were carried out at the level of the individual (Manel *et al.* 2003; Segelbacher *et al.* 2010; Storfer *et al.* 2010). We considered army ant dispersal in Monteverde to be affected by landscape variables in six basic ways: isolation by Euclidean distance (Model 1), isolation by land cover resistance (Model 2), isolation by elevation resistance (Model 3), isolation by combined land cover and elevation resistance (Model 4), isolation by elevation band resistance (increasing friction to disperse out of own elevation band, Model 5), and isolation by combined land cover and elevation band resistance (Model 6). To compare these models of dispersal, we constructed friction maps based on the five resistance models and calculated pairwise resistance distances among collection locations with the software CIRCUITSCAPE 3.5.4 (McRae 2006; McRae & Beier 2007; McRae *et al.* 2008; Goulson *et al.* 2011; Devitt *et al.* 2013). Resistance distances are based on circuit theory and simultaneously consider all possible pathways between nodes, with multiple or wider connecting corridors allowing greater gene flow (McRae 2006; McRae & Beier 2007; McRae *et al.* 2008).

For models 2, 4, & 6, we acquired multispectral satellite imagery (Landsat 7 ETM+, 30 m resolution) from the United States Geological Survey (USGS: earthexplorer.usgs.gov) and screened for images that had minimal cloud coverage over the Monteverde area (path/row: 15/53). We analyzed one image captured in wet season (6/15/2001) and one image from the following dry season (3/30/2002). For models 3 - 6, we downloaded a digital elevation model of the Monteverde area (SRTM, 90 m resolution) from the USGS website. All spatial analyses were performed in ArcGIS 10.0 (ESRI Inc., Redlands, CA, USA). We used images from 2001/2002 because army ant queens can live more than four years (Rettenmeyer 1963) and colony fission occurs approximately every three years in a stable population on Barro Colorado Island, Panama (Franks 1985; Kronauer 2009). Land cover in Monteverde in 2001-2002 is a result of development in the area since the 1950s and thus should affect the pattern of relatedness in 2006 if forest clearing inhibits emigration and dispersal. Because army ants migrate on average 78-81 m daily during the nomadic phase (Franks & Fletcher 1983; Willson *et al.* 2011), both Landsat images were pan-sharpened to 15 m resolution (ArcGIS 10.0 Resource Center, ESRI, 2011). We ran an Iso Cluster unsupervised classification on spectral bands 1 – 6 (ArcGIS 10.0 Resource Center, ESRI, 2011), and identified five final land cover classes (primary forest, secondary forest, edge, agriculture/pasture, urban/bare earth) based on satellite imagery, ecological data taken during sample collection and for previous studies (Kumar and O'Donnell 2009), and land use maps of the Monteverde area (Nadkarni & Wheelwright 2000). Classified land cover rasters were combined into one output raster by prioritizing more disturbed land cover classes (higher friction values) when input values disagreed and shrinking areas of missing data (ArcGIS 10.0 Resource Center, ESRI, 2011). We created friction maps by assigning values to land cover classes and elevation bands. Friction values are arbitrary and range from 0 to 100, but increasing friction values reflect greater habitat resistance to dispersal (McRae & Beier 2007; Goulson *et al.* 2011). Our six *a priori* models, one isolation by distance (IBD) and five isolation by resistance (IBR), were:

<u>Model 1 – Isolation by distance (IBD).</u> Euclidean distance was calculated as the straight line distance between collection points in a two-dimensional landscape, and the natural logarithm of this distance was used (as described for tests of sex-biased dispersal, above).

<u>Model 2 – Land cover.</u> *E. burchellii* rarely crosses open areas in lowland rainforest (Meisel 2006), but forages more frequently in open areas as elevation increases, possibly due to less drastic temperature differences between open and forested areas at high elevations (Kumar & O'Donnell 2009). Deforestation was found to inhibit (and mature forest promote) dispersal of *E. burchellii foreli* in Panama (Pérez-Espona *et al.* 2012a). Higher friction values were assigned to land classes of decreasing forest cover: primary forest = 10, secondary forest = 30, edge = 50, agriculture/pasture = 70, and urban/bare earth = 100, with 100 as a complete barrier to dispersal (Fig. 1). Grids consisted of 1041 X 1346 cells (15 m cell size).

<u>Model 3 – Elevation 1.</u> Army ant raids were found less frequently as elevation increased in Monteverde, Costa Rica (Kumar & O'Donnell 2009). The highest recorded observation of *E. burchellii* in the Monteverde area is 1622 m asl on the Pacific slope (Soare *et al.* 2011), though *E. burchellii* has been collected at 1670 m asl in the Talamanca mountain range in southern Costa Rica (J. T. Longino *pers*

comm.; antweb.org). Higher friction values were assigned to elevation bands of equal altitudinal width of increasing elevation: < 850 m = 10, 851 – 1050 m = 30, 1051 – 1250 m = 50, 1251 – 1450 m = 70, 1451 – 1650 m = 90, and > 1650 m = 100, with 100 as a complete barrier to dispersal (Fig. 2). Grids consisted of 173 X 200 cells (90 m cell size).

Model 4 – Land cover + Elevation 1. Friction maps from Models 2 and 3 were combined such that each cell of the resulting raster took on the maximum value of the input cells. This combined raster measures the additive effect of both land cover and elevation, and is equivalent to a multivariate approach (Spear *et al.* 2010). A friction value of 100 represents a complete barrier to dispersal. Grids consisted of 854 X 721 cells (15 m cell size).

Model 5 – Elevation 2. Though army ant encounter rate declines with elevation (Kumar & O'Donnell 2009), the Monteverde area consists of several Holdridge life zones (loosely correlated with elevation) (Holdridge 1967; Haber 2000), and colonies may be adapted to local environmental conditions. Colonies were assigned to elevation bands of at least 80 m in altitudinal width in an attempt to equalize colony samples sizes among bands: 950 – 1220 m (n = 5), 1221 – 1357 m (n = 5), 1358 – 1438 m (n = 11), 1439 – 1650 m (n = 4). Friction values were assigned such that a focal queen dispersing within her elevation band = 10, dispersing to a neighboring elevation band = 30, dispersing to a non-neighbor elevation band = 50, dispersing to a distant elevation band (separated by two or more bands) = 70, and > 1650 m = 100 (complete barrier to dispersal). Grids consisted of 173 X 200 cells (90 m cell size).

Model 6 – Land cover + Elevation 2. Friction maps from Models 2 and 5 were combined such that each cell of the resulting raster took on the maximum value of the input cells. This combined raster measures the additive effect of both land cover and elevation band, and is equivalent to a multivariate approach (Spear *et al.* 2010). A friction value of 100 represents a complete barrier to dispersal. Grids consisted of 854 X 720 cells (15 m cell size).

Because queens disperse terrestrially, but males disperse by flying, we predict landscape processes would affect colony emigrations and therefore patterns of queen dispersal. Thus we evaluated isolation by resistance scenarios on queen genotypes. We assume that males, which likely fly across the waters of the Panama Canal and Chagres River (Berghoff *et al.* 2008; Pérez-Espona *et al.* 2012a), are able to cross deforested areas as well. Pairwise relatedness among queens was regressed on pairwise resistance distances for Models 2-6 in SPAGeDi as described for Euclidean distance above. Because we were interested in comparing dispersal scenarios on queen genotypes, the pairwise relationship coefficient (r_{ij}: Queller & Goodnight 1989) is an appropriate genetic distance measure for dispersal within a single caste (Sundström *et al.* 2003; Hazlitt *et al.* 2004), but to be consistent results based on the pairwise kinship coefficient (F_{ij}: Loiselle *et al.* 1995) are provided as well. In a continuous two-dimensional landscape, resistance distance is expected to scale linearly with genetic similarity (McRae 2006; McRae *et al.* 2008). Because we regressed the same genetic similarity value (r_{ij} among queens), coefficients of determination (r^2) were compared among the various models to determine which predictor distance (Model) accounted for the most variation in the genetic distance among queens (highest r^2 value) (McRae 2006; Pérez-Espona *et al.* 2008; Devitt *et al.* 2013). To account for testing six *a priori* distance models we adjusted our alpha level with the Benjamini and Yekutieli false discovery rate procedure (B-Y FDR: Benjamini &Yekutieli 2001; Narum 2006; Latch *et al.* 2011). Due to the challenge of assigning friction values when specific landscape effects are unknown (Spear *et al.* 2010), a range of friction values or resistance distances was tested for each model (see Table S2, Supporting information, for details).

To determine if habitat variables affect queen dispersal after removing any effect of Euclidean distance, we performed partial Mantel tests in R 2.14.1 (R Development Core Team 2011) using the package ECODIST 1.2.5 (Goslee & Urban 2007) and obtained two-tailed *P* values based upon 10000 permutations of pairwise genetic similarity among all pairs (Goslee & Urban 2007; Goulson *et al.* 2011;

Short Bull *et al.* 2011). Partial Mantel tests are a widespread method for detecting effects of landscape on genetic structure while removing any effects of Euclidean distance (McRae & Beier 2007; Storfer *et al.* 2010; Goulson *et al.* 2011; Short Bull *et al.* 2011), and this method is appropriate for use on distance matrices (Legendre & Fortin 2010). We report uncorrected *P* values for all possible partial Mantel tests (including inverse partial Mantel tests) and adjusted our alpha level using the B-Y FDR procedure to account for these multiple tests (Benjamini &Yekutieli 2001; Narum 2006; Latch *et al.* 2011). Testing all possible Mantel and partial Mantel tests in this manner is equivalent to a causal modeling framework (Cushman *et al.* 2006; Cushman & Landguth 2010a).

Performing multiple partial Mantel tests can lead to inflation of the Type I error rate (Legendre & Legendre 1998; Goslee & Urban 2007; Balkenhol *et al.* 2009), especially when the predictors (distance measures) are highly correlated (Cushman & Landguth 2010a). Therefore, we also performed a random forests analysis to confirm the reliability of our results. A random forests (RF) analysis constructs many independent bifurcating trees with a bootstrap sample, splitting each node with the best predictor variable from a randomly chosen subset of predictors (Breiman 2001; Liaw & Wiener 2002; Thomassen *et al.* 2010). RF then predicts the remaining observations (out-of-bag, OOB) with the constructed trees, and compares the OOB prediction error to that obtained when the OOB data is randomly permuted (Liaw & Wiener 2002; Prasad *et al.* 2006; Cutler *et al.* 2007; Murphy *et al.* 2010; Thomassen *et al.* 2010). This approach is a robust means for determining variable importance, as RF makes no *a priori* assumptions about the relationship among variables and is insensitive to collinearity and autocorrelation (Breiman 2001; Liaw & Wiener 2002; Prasad *et al.* 2006; Cutler *et al.* 2007; Thomassen *et al.* 2010). We ran a RF analysis in the randomForest package (Liaw & Wiener 2002) in R 2.14.1 (R Development Core Team 2011). We predicted the pairwise relationship coefficient (r_{ij}) and pairwise kinship coefficient (F_{ij}) among queens using pairwise Euclidean and resistance distances with 5000 trees (number of bootstrap iterations) split by the best of 2 randomly chosen predictors at each node (m_{try}), where 36% of the observations were withheld for each tree (OOB sample) (Liaw & Wiener 2002; Murphy *et al.* 2010). We repeated the analysis with random variable subsets of size 4 and 1 (Liaw & Wiener 2002), though RF is generally insensitive to different values of this parameter, m_{try} (Liaw & Wiener 2002; Cutler *et al.* 2007). In RF regression analyses, variable importance is measured as the normalized difference in mean square error (MSE) when predicting the OOB data when that variable is included as observed versus when it is randomly permuted (Breiman 2001; Liaw & Wiener 2002; Murphy *et al.* 2010). Larger differences represent variables that are more important to the regression. Variable importance can also be determined by the increase in accuracy, which is defined as the reduction in node impurities when splitting on the variable, as measured by residual sum of squares (Breiman 2001; Liaw & Wiener 2002).

Results

Quality of genetic data

After initially genotyping 421 workers, two workers were removed for having genotypes incompatible with the majority matriline (i.e. likely the daughter of a previously replaced queen: Kronauer *et al.* 2006), and two workers due to poor amplifications (missing data at four of seven and five of seven loci, respectively). We also removed four workers from the dataset for likely 2-bp mutations (two at *Eb24* and two at *Eb51*); taking these mutations into account would have made the genotypes consistent with the majority matriline. This finding reflects the previously reported high mutation rate of these loci (Kronauer *et al.* 2006). All remaining workers (n = 413, 13-20 workers per colony) were genotyped at five or more loci (390 workers had full genotypes at all loci).

Population descriptive statistics

COLONY reconstructed 25 female and 246 male genotypes contributing to workers from the 25 collections; thus each collection used in the genetic analysis was a unique colony. COLONY results were replicated with additional runs with different random number seeds. The average number of mates per queen was 9.8 +/- 0.4 SE (range: 5 – 14) and the effective mating frequency (m_e: Nielsen *et al.* 2003) was 11.0 +/- 1.5 SE. The effective mating frequency (m_e) of army ant queens did not significantly differ among populations studied to date: Barro Colorado Island, Panama (12.9 +/- 1.1 SE, Kronauer *et al.* 2006), Henri Pittier National Park, Venezuela (9.4 +/- 0.2 SE, Kronauer & Boomsma 2007), and Chiapas, Mexico (12.7 +/- 0.5 SE, Jaffé *et al.* 2009) (Kruskal-Wallis ANOVA $X^2 = 5.77$, $P = 0.12$).

F_{Iq}, a measure of inbreeding in queens, was not significantly different from zero (-0.02 +/- 0.05 SE, $P = 0.503$). Additionally, F_{Iq} for each locus was also not significantly different from zero (each $P > 0.05$). We did not detect any significant linkage between any pairs of loci for the queen genotypes (each $P > 0.0024$, the nominal P after Bonferroni adjustment for multiple comparisons). Males mated to the same queen were related: pairwise kinship among a queen's mates, F_{mm}, was significantly greater than zero (0.0553 +/- 0.0076 SE, $P < 0.0001$). The two measures of genetic similarity between queens, the pairwise kinship coefficient (F_{ij}: Loiselle *et al.* 1995) and the pairwise relationship coefficient (r_{ij}: Queller & Goodnight 1989), were highly correlated ($r = 0.924$, $P < 0.0001$).

Power analysis

Both the pairwise kinship coefficient (F_{ij}) and the pairwise relationship coefficient (r_{ij}) significantly predicted true pairwise relatedness (r_{true}) among genotypes simulated using allele frequencies from Monteverde (*Adj* $R^2 = 0.474$, $P < 0.0001$; and *Adj* $R^2 = 0.414$, $P < 0.0001$; respectively; Fig. S1, Supporting information). Values of the pairwise kinship and relationship coefficients were significantly greater for the highest relatedness class ($r_{true} = 0.5$) than for the other classes of relatedness (Tukey HSD $P < 0.0001$ in each case, Fig. S1). Both genetic distance measures for intermediate levels of relatedness ($r_{true} = 0.25$

and r_{true} = 0.125) were significantly different from the lowest levels of relatedness (r_{true} = 0.0313 and r_{true} = 0, Tukey HSD $P < 0.05$ in all cases, Fig. S1).

Dispersal

The regression of the pairwise kinship coefficient (F_{ij}) on the natural logarithm of Euclidean distance (Model 1) showed no significant isolation by distance for any caste over the spatial extent of 9.3 km measured in Monteverde (queens: $b = -0.002$, $r^2 = 0.0004$, $P = 0.687$; males: $b = -0.0007$, $r^2 < 0.0001$, $P = 0.684$; workers: $b = 0.0003$, $r^2 < 0.0001$, $P = 0.932$; Table 1, Fig. 3). There was also no significant spatial genetic structure over distance categories up to 7 km, 4 km, 2 km, 1 km or 500 m for any caste (Table 1).

In the 15 colonies sampled by Pérez-Espona *et al.* (2012a, 2012b) in San Lorenzo, Panama, pairwise kinship (F_{ij}) among queens significantly declined with the natural logarithm of Euclidean distance over 16.1 km ($b = -0.021$, $r^2 = 0.0640$, $P = 0.018$; Table 1, Fig. 4). Pairwise kinship (F_{ij}) among males also significantly declined over the same distance ($b = -0.014$, $r^2 = 0.0060$, $P = 0.005$). When pairwise kinship was regressed over a distance range restricted to 9.3 km (the maximum pairwise distance sampled in Monteverde, Costa Rica), there was still evidence of spatial genetic structuring in both sexes (queens: $b = -0.024$, $r^2 = 0.0652$, $P = 0.033$; males: $b = -0.011$, $r^2 = 0.0033$, $P = 0.047$; Table 1).

Effects of landscape

To compare effects of landscape variables on colony dispersal, we regressed the pairwise relationship coefficient (r_{ij}) among queens on resistance distances. Pairwise relatedness among queens significantly declined with land cover resistance (Model 2: $b = -0.0020$, $r^2 = 0.0469$, $P = 0.005$, Table 2). Queen pairwise relatedness significantly increased with increasing elevation resistance (Model 3: $b = 0.0025$, $r^2 = 0.0247$, $P = 0.015$). There was no significant effect of elevation band (Model 5: $b = 0.0024$, $r^2 = 0.0067$, $P = 0.202$) or elevation combined with land cover (Model 4: $b = -0.0006$, $r^2 = 0.0052$, $P = 0.323$; and

Model 6: b = -0.0013, r^2 = 0.0232, P = 0.039) on pairwise relatedness among queens. There was no significant isolation by resistance for the pairwise kinship coefficient (F_{ij}) in any of our resistance models over the spatial extent measured (Table 2). However, land cover (Model 2) accounted for the greatest amount of variation in pairwise kinship relative to the other resistance distances tested (b = -0.0004, r^2 = 0.0093, P = 0.080). Tests of the effects of different friction values or resistance distances on model results revealed few qualitative changes. Statistical significance was lost in two models predicting the pairwise relationship coefficient (r_{ij}) among queens: when the least vegetated class (urban/bare earth) and when the highest elevations (> 1650 m asl) were not considered to be complete barriers to dispersal (Models 2.g and 3.f: Table S2, Supporting information).

Some of the distance measures were significantly correlated with each other (Table 3). After controlling for Euclidean distance, land cover (Model 2) significantly predicted values of the pairwise relationship coefficient (r_{ij}): pairwise relatedness among queens decreased with increasing land cover resistance (r = -0.2252, P = 0.001, Table 4). Euclidean distance remained a non-significant predictor of pairwise relatedness among queens after controlling for land cover resistance (r = 0.0793, P = 0.226). After controlling for Euclidean distance, elevation (Model 3) also remained a significant predictor of the pairwise relationship coefficient such that relatedness among queens increased with increasing elevation (r = 0.1930, P = 0.008). Euclidean distance remained a non-significant predictor of queen relatedness after controlling for elevation resistance (r = -0.1229, P = 0.078). In a *post hoc* partial Mantel test controlling for land cover resistance (Model 2), the significance of elevation (Model 3) as a predictor of pairwise queen relatedness (r_{ij}) disappeared (r = 0.1172, P = 0.098). Alternatively, land cover resistance (Model 2) remained a significant predictor of pairwise relatedness (r_{ij}) among queens after controlling for elevation resistance (Model 3), in a second *post hoc* partial Mantel test (r = -0.1902, P = 0.008).

Resistance distances based on models 4 and 5 did not significantly predict values of the pairwise relationship coefficient (r_{ij}) after controlling for Euclidean distance (Table 4). Land cover combined with elevation band (Model 6) significantly predicted queen relatedness after controlling for Euclidean distance (r = -0.1941, P = 0.008), but the original regression for Model 6 was not significant (Table 2). There were no significant partial Mantel tests predicting values of the pairwise kinship coefficient (F_{ij}, Table 4).

In random forests (RF) analyses, land cover ranked highest in variable importance for predicting both the pairwise relationship coefficient (r_{ij}) and pairwise kinship coefficient (F_{ij}) among queens (Fig. 5). Land cover remained the highest ranked for variable importance in analyses with randomly chosen variable subsets (m_{try}) of size 4 and 1 (Fig. S2, S3, Supporting information).

Discussion

Our aims were to examine patterns of dispersal among different sexes in army ants in Monteverde, Costa Rica, and to determine whether forest clearing and elevation affected gene flow. The mating frequency of Monteverde army ant queens was comparable with other studies. We did not detect any evidence for reduced diversity within individuals, nor linkage disequilibrium, suggesting that there is no inbreeding in this population. Thus either gene flow is maintained in the Monteverde area or current levels of forest clearing and development have not yet restricted access to mates nor generated inbreeding.

Dispersal patterns across castes

We found no spatial genetic structure in queens (which disperse by walking) or males (which disperse by flying) up to 9.3 km in Monteverde, Costa Rica. However, in most regressions between genetic similarity and Euclidean distance, the slope of the decline of genetic relatedness was of greater magnitude for queens than for males, potentially indicating undetected queen spatial genetic structure within the distances measured. Berghoff *et al.* (2008) found weak but significant genetic differentiation among subpopulations separated by 5 – 10 km in the Panama Canal Zone, suggesting a dispersal limitation for one or both sexes. Contrary to the results from Monteverde, spatial genetic structure in both sexes was readily detected from samples collected in San Lorenzo, Panama (Pérez-Espona *et al.* 2012b). The decline in male pairwise relatedness across the entire spatial extent in San Lorenzo was more significant than that of queens (as indicated by a lower *P* value), but this may simply represent increased power to detect spatial genetic structuring (i.e. more male pairwise comparisons). The slope of the regression of queen pairwise relatedness on distance was more negative than that of males in San Lorenzo, suggesting that there was stronger spatial genetic structuring in queens. Comparing pairwise relatedness estimates among individuals at nuclear loci across the sexes supported previously reported differences in nuclear and mitochondrial DNA differentiation (Pérez-Espona *et al.* 2012a), demonstrating the utility of the individual-based approach in detecting sex-biased dispersal.

The spatial extent of the current study may have been insufficient for detecting isolation by distance. We detected isolation by distance in queens and males sampled over 16.1 km in Panama (Pérez-Espona *et al.* 2012b), suggesting that larger geographic distances may be important for studying sex-biased dispersal. However, the distance over which colonies were sampled in Monteverde represents a longer distance than most army ant dispersal estimates (Berghoff *et al.* 2008; Jaffé *et al.* 2009). In other ant species examined to date, dispersal distance of winged reproductives has been estimated to be up to 2 km (Vogt *et al.* 2000; Doums *et al.* 2002; Clemencet *et al.* 2005; Hardy *et al.* 2008). Furthermore, regressions of queen and male pairwise relatedness restricted to a distance of 9.3 km in San Lorenzo displayed the same significant patterns of queen and male spatial genetic structuring. Thus the spatial extent of 9.3 km may be sufficient to detect the same patterns in Monteverde, if they occur in the population.

The spatial sampling scheme in Monteverde was a mix of systematic trail walks for another study (Kumar & O'Donnell 2009) and opportunistic encounters. Because forest fragments, trails, and collections were not uniformly distributed across the study area, we risk losing power to detect relatedness patterns (Oyler-McCance *et al.* 2013). However, sampling effort was distributed among multiple sites and collection points along trails represent a linear sampling scheme with an assumption of even distribution of potential observations of army ant colonies. Spatial genetic structuring was successfully detected in lowland Panama from opportunistic collections (Pérez-Espona *et al.* 2012a, 2012b). In simulations, random, linear, and systematic grid sampling schemes performed equally well in detecting landscape effects on gene flow (Oyler-McCance *et al.* 2013). We also maximized the number of pairwise comparisons within distance intervals for the autocorrelation analyses (Storfer *et al.* 2007). Undersampling of pairs of colonies in the maximum distance interval may have introduced bias into the computation of the average genetic similarity at the greatest distances. Small sample sizes are known to reduce the power to detect spatial genetic structure (Banks & Peakall 2012; Landguth *et al.* 2012), but the number of colonies sampled in Monteverde exceeded that of San Lorenzo (Pérez-Espona *et al.* 2012a), and thus also appears sufficient to detect spatial genetic structuring in Monteverde. We were confident that colonies used in genetic analyses were all unique. If a colony had been re-sampled, the same queen genotype would have been reconstructed in COLONY (Jones & Wang 2010) and shared patrilines among workers from different collections would have been observed (Kronauer *et al.* 2006; Kronauer & Boomsma 2007).

The slopes of the regressions of pairwise kinship on distance are over an order of magnitude more negative for San Lorenzo than for Monteverde. The respective geographies of the two sampled populations may have led to differences in levels of spatial genetic structuring. San Lorenzo is located on a peninsula created by the construction of the Panama Canal (see Fig. 1 in Pérez-Espona *et al.* 2012a) and Monteverde consists of multiple fragments of formerly continuous forest (Nadkarni & Wheelwright

2000). An army ant population on a peninsula may be more likely to display strong spatial genetic structuring than those in sites unconfined by water. On the other hand, colony emigration patterns were not observed to differ between a confined population on Barro Colorado Island and populations in continuous forest (Franks & Fletcher 1983; Willson *et al.* 2011; but see Califano & Chaves-Campos 2011). The relatively recent history of development in the Monteverde area (since the 1950s: Nadkarni & Wheelwright 2000), as compared to the construction of the Panama Canal (1910s: Berghoff *et al.* 2008), may mean that the natural population in Monteverde is not yet at equilibrium (Hardy & Vekemans 1999) or insufficient generations have passed to create isolation by distance (Vekemans & Hardy 2004; Landguth *et al.* 2012). However, spatial genetic structure and sex-biased dispersal have been detected in simulations after a single generation (Landguth *et al.* 2010; Banks & Peakall 2012). Alternatively, dispersal patterns may differ between populations due to ecology or evolutionary histories (Spear *et al.* 2010). The Monteverde and San Lorenzo populations actually consist of morphologically distinct subspecies (*E. b. parvispinum* and *E. b. foreli*, respectively), and any behavioral differences may contribute to dissimilar patterns of spatial genetic structuring.

The power to discriminate among levels of pairwise relatedness may be limited by the number of loci employed (Landguth *et al.* 2012), though the same number of loci were amplified in both army ant populations (Pérez-Espona *et al.* 2012a). However, simulated genotypes based on population allele frequencies demonstrated that both measures of genetic similarity we utilized (pairwise kinship coefficient, F_{ij}, and pairwise relationship coefficient, r_{ij}) were able to discriminate among high, intermediate, and low levels of relatedness. Our analysis of dispersal of army ants is based on reconstructed genotypes and our ability to reconstruct male genotypes is limited by the number of workers sampled. However, sperm mixing in the queen spermatheca (Kronauer *et al.* 2006) likely ensures that the males sampled are not a biased representation of the population.

The use of maternally-inherited markers (i.e. mitochondrial DNA, mtDNA) may provide greater power to detect dispersal patterns across sexes. In San Lorenzo, Panama, Pérez-Espona *et al.* (2012a) found mitochondrial differentiation to be correlated with Euclidean distance and resistance distances based on major water features such as the Chagres River. On the other hand, the authors of that study found no effects of more recent deforestation on mtDNA differentiation (Pérez-Espona *et al.* 2012a). Berghoff *et al.* (2008) found no mitochondrial differentiation in a 525-bp region of *COI* in *E. burchellii* between BCI and mainland Panama after nearly 100 years of separation (more than 1 km distant), but they did find matriline separation by the historic route of the Chagres River. Indeed, mitochondrial DNA may be most appropriate for inferring historical gene flow and nuclear DNA (e.g. microsatellite loci) for inferring contemporary gene flow (Foitzik *et al.* 2009; Anderson *et al.* 2010; Storfer *et al.* 2010; Wang 2010, 2011a). Therefore our examination of sex-biased dispersal and the effects of landscape on spatial genetic structure of nuclear loci provided insight into contemporary gene flow in the region.

We did not find spatial genetic structuring in either sex in this dependent founding ant species in Monteverde, Costa Rica. Regular colony emigrations may facilitate matrilineal gene flow in *E. burchellii.* If a colony continued marching over the landscape for the queen's lifetime (at least 4 years: Rettenmeyer 1963), then summed migrations may separate related queens by several kilometers. Successive statary bivouacs are located about 500 m apart, which is greater than the expected distance if a colony was performing a random walk during the nomadic phase (Franks & Fletcher 1983; Willson *et al*. 2011). Colonies also follow a single compass bearing during entire nomadic phases (Franks & Fletcher 1983; Willson *et al*. 2011; but see Califano & Chaves-Campos 2011). If a colony pursued the same compass bearing for four years, and moved 500 m in each nomadic phase, it would travel approximately 21 km. Unfortunately, long-term data (over multiple reproductive phases) on single colonies in continuous habitat is lacking. Long-term observations in continuous habitat, or repeat genetic sampling, may elucidate lifetime movements of *E. burchellii* colonies.

Long distance dispersal is favored under conditions of environmental heterogeneity (Johnson & Gaines 1990). *Eciton burchellii* exhaust resources locally (Franks 1982; Otis *et al.* 1986), migrate to new foraging grounds (Franks & Fletcher 1983; Kaspari & O'Donnell 2003), and specialize on high prey-density patches (Kaspari *et al.* 2011). Such resource heterogeneity, combined with low emigration costs (e.g., low inter-colony aggression: Swartz 1997; Willson *et al.* 2011), may have selected for high queen dispersal rate and distance in *Eciton burchellii* in continuous habitat.

Effects of landscape on dispersal

Although a simple isolation by distance model did not explain queen relatedness in Monteverde, we found evidence for genetic isolation by landscape resistance in *Eciton burchellii*. Resistance distances based on land cover accounted for the greatest amount of variation in pairwise kinship (relative to the other resistance distances tested) and significantly predicted pairwise relatedness after controlling for any effects of Euclidean distance and elevation. Testing a range of friction values and resistance distances for each model did not qualitatively change the results. A random forests analysis further showed that resistance distance based on land cover was the most important variable in predicting pairwise queen relatedness. Therefore summed migrations over a colony's lifetime may contribute to gene flow in this species and forest clearing inhibits dispersal in our montane study site. The effects of land cover on army ant dispersal in Monteverde agree with those found in a study in San Lorenzo: resistance distances based on deforestation explained most of the pairwise differentiation among colonies at nuclear loci (Pérez-Espona *et al.* 2012a).

We observed a discrepancy in the regressions of the two measures of genetic similarity on landscape resistance. The measures are closely linked: $r_{ij} = k_jF_{ij}/(1 + (k_j – 1)F_{Ij})$, where k_j is the ploidy level and F_{Ij} is the inbreeding level of individual j (Hardy *et al.* 2008) and were highly correlated among queens. However, only patterns of relatedness based on the pairwise relationship coefficient (r_{ij}) were significantly predicted by resistance distances based on land cover. The pairwise relationship coefficient (r_{ij}) does not account for inbreeding, unlike the pairwise kinship coefficient (F_{ij}) (Hardy *et al.* 2008; Sanllorente *et al.* 2010). The trend of outbreeding in queens in the Monteverde population (though not significant) may influence the estimation of pairwise kinship for outbred queens and could lead to disagreements in the results of regressions based on the two measures.

The friction maps were based on satellite data and therefore the models of isolation by resistance may not have captured abiotic factors that could be relevant to army ant dispersal such as precise Holdridge life zone (Holdridge 1967; Haber 2000), microclimatic differences (Meisel 2006; O'Donnell & Kumar 2006; Soare *et al.* 2011), or fine-scale topography (Mullen *et al.* 2010; Murphy *et al.* 2010; Angelone *et al.* 2011; Latch *et al.* 2011). We also assumed no other barriers to dispersal (e.g. roads, swamps, streams), though we have observed *E. burchellii* colonies crossing dirt roads and streams (T.S., A.K., and S.O'D. pers obs.). In San Lorenzo, Pérez-Espona *et al.* (2012a) did not find effects of roads or a swamp on army ant dispersal, though streams were found to both facilitate dispersal (as measured by mtDNA differentiation) and inhibit dispersal when interacting with deforestation (as measured by microsatellite differentiation). Past ecological studies have found that exposed areas limit colony movements (Meisel 2006), though the effect declines with elevation (Kumar & O'Donnell 2009). A land cover classification captures differences in forest cover (Cihlar 2000), and because *Eciton burchellii* is a forest interior species, resistance distances based on land cover may be most relevant when examining queen dispersal in this species.

Resistance distances based on elevation were positively correlated with queen pairwise relatedness, suggesting that colonies may preferentially disperse to or through high elevations. However, in the Monteverde area, higher elevation areas have more primary forest; elevation resistance was negatively, though not significantly, correlated with land cover resistance. Because the significance of elevation as a predictor of queen relatedness disappeared after controlling for land cover, elevation may neither inhibit nor encourage colony dispersal. Our collections in Monteverde did not capture all the variability in the elevation range of *Eciton burchellii* (Watkins 1976), and thus may have reduced our power to detect effects of elevation on army ant dispersal (Short Bull *et al.* 2011). Furthermore, we did not sample elevation variability in the absence of forest fragmentation. Kronauer *et al.* (2010) found spatial genetic structuring of mitochondrial DNA in the African army ant *Dorylus molestus* over a 22 km transect in Mt. Kenya Forest Reserve, Kenya (over a 1200 m elevation range). Although occupying similar trophic niches, the timing and direction of *D. molestus* emigrations are more random than those of *E. burchellii* (Gotwald 1995; Schöning *et al.* 2005), and could lead to relatively greater spatial genetic structuring in *D. molestus.* Though power also decreases with coarser grain sizes (Storfer *et al.* 2007; Cushman & Landguth 2010b), a 90 m grain size for elevation is still less than the average distance covered by a colony during the entire nomadic phase (*c.* 500 m: Franks & Fletcher 1983; Willson *et al.* 2011), and thus appears to be sufficient to detect effects of elevation on dispersal within the range sampled (Anderson *et al*. 2010). We may not have had sufficient power to examine army ant dispersal among elevation bands, since one band contained nearly half of the samples and many samples were collected in adjacent locations. However, the non-significant positive trend between resistance distances based on elevation and elevation band with genetic relatedness suggest that land cover seems to be the only examined landscape variable that inhibits army ant dispersal in the Monteverde area.

Eciton burchellii has a broad latitudinal and elevational range (Watkins 1976). Raiding behavior (O'Donnell & Kumar 2006; Kumar & O'Donnell 2009) and bivouac site selection (Soare *et al.* 2011) change with elevation. If queens are capable of migrating long distances (including hundreds of meters in elevation) over their lifetimes, then colonies may encounter variation in temperature regimes and life zone ecology (Holdridge 1967; Haber 2000). Perhaps colony-level behaviors are phenotypically flexible, such that colonies respond to local environmental conditions. Experimental colony relocations (e.g. Franks & Fletcher 1983) could be used to test this hypothesis.

The negative effects of forest clearing on army ant mobility are known to decline at higher elevations, as foraging activity in open areas increases with elevation (Kumar & O'Donnell 2009). Thus the effects of land cover on emigration may be more pronounced in lowland forests (Pérez-Espona *et al.* 2012a). Army ants avoid open areas in lowlands (Meisel 2006), and populations are vulnerable to extinction in forest fragments (Partridge *et al.* 1996; Boswell *et al.* 1998; Meisel 2004). We recommend maintaining habitat connectivity to facilitate *Eciton* gene flow across the landscape. Future research should address which habitat types and corridor widths permit *Eciton* gene flow among isolated populations, and what levels of gene flow are required to allow population persistence across the latitudinal and elevational range of these top predators. Supporting army ant populations would increase local biodiversity (Rettenmeyer *et al.* 2011), especially for ant-following birds (Meisel 2004; Kumar & O'Donnell 2007). Increasing development and climate change may exacerbate the inhibition of army ant dispersal by forest clearing. Therefore re-evaluations of the effect of habitat fragmentation on dispersal of this keystone species are vital.

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Data accessibility

Microsatellite genotypes and collection locations uploaded as DRYAD entry doi:10.5061/dryad.j33k3.

Tables

Table 1 Pairwise genetic similarity (kinship coefficient, F_{ij}) within castes is not significantly predicted by Euclidean distance over any restricted distance in *E. burchellii parvispinum* (Monteverde, Costa Rica) but is for *E. burchellii foreli* (San Lorenzo, Panama) within 16.1 km, 9.3 km, 7 km (males only), and 0.5 km. Sample size (*N*) is the number of pairwise comparisons among individuals (excluding within-colony comparisons) over designated spatial extent. Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values represent significant relationships.

		Monteverde, Costa Rica				**San Lorenzo, Panama**			
Caste	**Spatial extent (km)**	***N***	***b***	r^2	***P***	***N***	***b***	r^2	***P***
queens	0-16.1	-	-	-	-	**105**	**-0.0207**	**0.0640**	**0.018**
males		-	-	-	-	**22685**	**-0.0137**	**0.0060**	**0.005**
workers		-	-	-	-	-	-	-	-
queens	0-9.3	300	-0.0022	0.0004	0.687	**69**	**-0.0243**	**0.0652**	**0.033**
males		28999	-0.0007	< 0.0001	0.684	**14458**	**-0.0111**	**0.0033**	**0.047**
workers		81794	0.0003	< 0.0001	0.932	-	-	-	-
queens	0-7	266	-0.0059	0.0023	0.415	54	-0.0231	0.0479	0.076
males		25409	0.0008	< 0.0001	0.764	**11260**	**-0.0154**	**0.0056**	**0.032**
workers		72901	0.0003	< 0.0001	0.942	-	-	-	-
queens	0-4	193	-0.0054	0.0014	0.566	26	-0.0375	0.0587	0.089
males		18302	-0.0008	< 0.0001	0.755	5458	-0.0139	0.0030	0.204
workers		53413	-0.00003	< 0.0001	0.959	-	-	-	-
queens	0-2	98	0.0111	0.0043	0.493	12	-0.0080	0.0015	0.703
males		9304	-0.0026	0.0001	0.590	2518	-0.0057	0.0004	0.662
workers		26686	-0.0020	0.0002	0.702	-	-	-	-
queens	0-1	38	-0.0032	0.0003	0.900	**7**	**0.0812**	**0.0769**	**0.046**
males		3492	-0.0003	< 0.0001	0.973	**1492**	**0.0806**	**0.0424**	**0.003**
workers		10620	-0.0084	0.0025	0.382	-	-	-	-
queens	0-0.5	11	-0.0025	0.0003	0.988	1	-	-	-
males		1105	-0.0002	< 0.0001	0.993	221	-	-	-
workers		2998	-0.0302	0.0159	0.237	-	-	-	-

Table 2 Resistance distances based on land cover and elevation significantly predict pairwise genetic similarity (relationship coefficient, r_{ij}) among queens. Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values represent significant relationships (B-Y FDR adjusted alpha = 0.0161).

		pairwise relationship (r_{ij})			**pairwise kinship (F_{ij})**		
Caste	**Model**	***b***	**r^2**	***P***	***b***	**r^2**	***P***
queens	1. ln(Euclidean)	0.0119	0.0023	0.475	-0.0022	0.0004	0.687
males	1. ln(Euclidean)	--	--	--	-0.0007	0.00001	0.684
workers	1. ln(Euclidean)	0.0068	0.0007	0.469	0.0003	0.00001	0.932
queens	2. Land cover	**-0.0020**	**0.0469**	**0.005**	-0.0004	0.0093	0.080
queens	3. Elev1	**0.0025**	**0.0247**	**0.015**	0.0003	0.0019	0.440
queens	4. Land + Elev1	-0.0006	0.0052	0.323	-0.0002	0.0044	0.232
queens	5. Elev2	0.0024	0.0067	0.202	0.0004	0.0007	0.681
queens	6. Land + Elev2	-0.0013	0.0232	0.039	-0.0003	0.0060	0.161

Table 3 Pairwise distance measures among sample locations are correlated. Correlation coefficients (*r*) lie above the diagonal while significance values (*P*, two-tailed) lie below. Bold values represent significant correlations (Bonferroni adjusted alpha level = 0.0033).

	1. ln(Eucl)	**2. Land cover**	**3. Elev1**	**4. Land + Elev1**	**5. Elev2**	**6. Land + Elev2**
1. ln(Eucl)	.	0.1349	**0.7826**	**0.7108**	**0.7734**	**0.4475**
2. Land cover	0.2804	.	-0.2089	**0.6733**	0.0045	**0.9010**
3. Elev1	**0.0001**	0.2055	.	**0.5270**	**0.6138**	0.0535
4. Land + Elev1	**0.0001**	**0.0001**	**0.0002**	.	**0.4751**	**0.8212**
5. Elev2	**0.0001**	0.9729	**0.0001**	**0.0003**	.	**0.4131**
6. Land + Elev2	**0.0002**	**0.0001**	0.7447	**0.0001**	**0.0005**	.

Table 4 Resistance distances based on land cover and elevation significantly predicted pairwise genetic similarity (relationship coefficient, r_{ij}) after controlling for Euclidean distance. Each partial Mantel test was modeled as Y ~ X_1 | X_2 and measured the correlation of X_1 & Y after controlling for (given) X_2. Correlation coefficient (*r*) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values represent significant relationships (B-Y FDR adjusted alpha = 0.0139).

Y	**Model**	**X_1**	**X_2**	***r***	***P***
r_{ij}	**2**	**Land cover**	**ln(Eucl)**	**-0.2252**	**0.001**
		ln(Eucl)	Land cover	0.0793	0.226
	3	**Elev1**	**ln(Eucl)**	**0.1930**	**0.008**
		ln(Eucl)	Elev1	-0.1229	0.078
	4	Land cover + Elev1	ln(Eucl)	-0.1502	0.047
		ln(Eucl)	Land cover + Elev1	0.1404	0.039
	5	Elev2	ln(Eucl)	0.0718	0.252
		ln(Eucl)	Elev2	-0.0254	0.682
	6	**Land cover + Elev2**	**ln(Eucl)**	**-0.1941**	**0.008**
		ln(Eucl)	Land cover + Elev2	0.1307	0.053
F_{ij}	2	Land cover	ln(Eucl)	-0.0948	0.077
		ln(Eucl)	Land cover	-0.0061	0.916
	3	Elev1	ln(Eucl)	0.0943	0.080
		ln(Eucl)	Elev1	-0.0857	0.115
	4	Land cover + Elev1	ln(Eucl)	-0.0753	0.179
		ln(Eucl)	Land cover + Elev1	0.0402	0.484
	5	Elev2	ln(Eucl)	0.0650	0.241
		ln(Eucl)	Elev2	-0.0623	0.273
	6	Land cover + Elev2	ln(Eucl)	-0.0773	0.156
		ln(Eucl)	Land cover + Elev2	0.0177	0.754

Figures

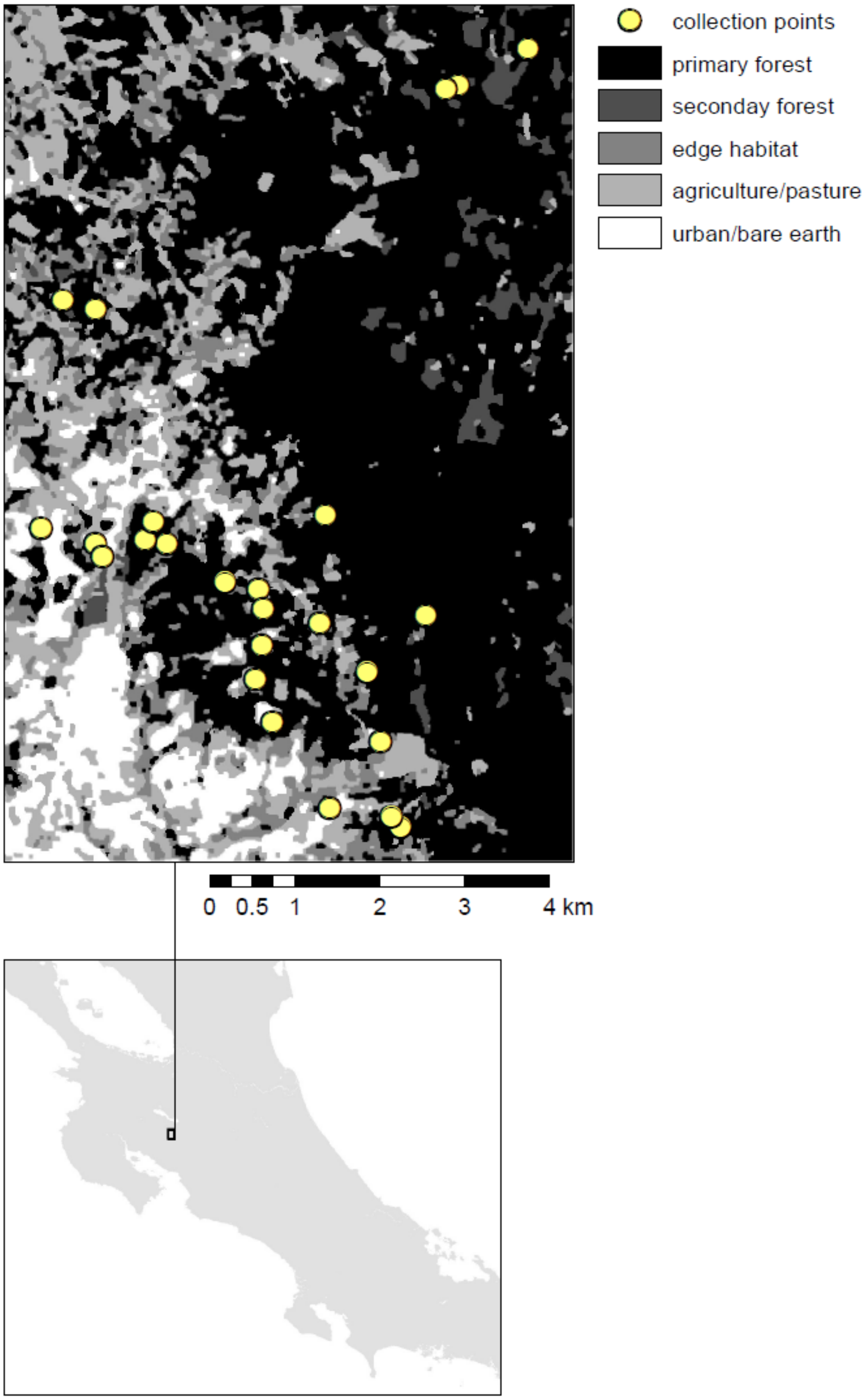

Fig. 1 Location of study site and map of land cover classes with locations of sampled colonies. Five land cover classes (friction values) are primary forest (10), secondary forest (30), edge habitat (50), agriculture/pasture (70), urban/bare earth (100). Friction values are estimates of habitat resistance with 100 being a complete barrier to dispersal.

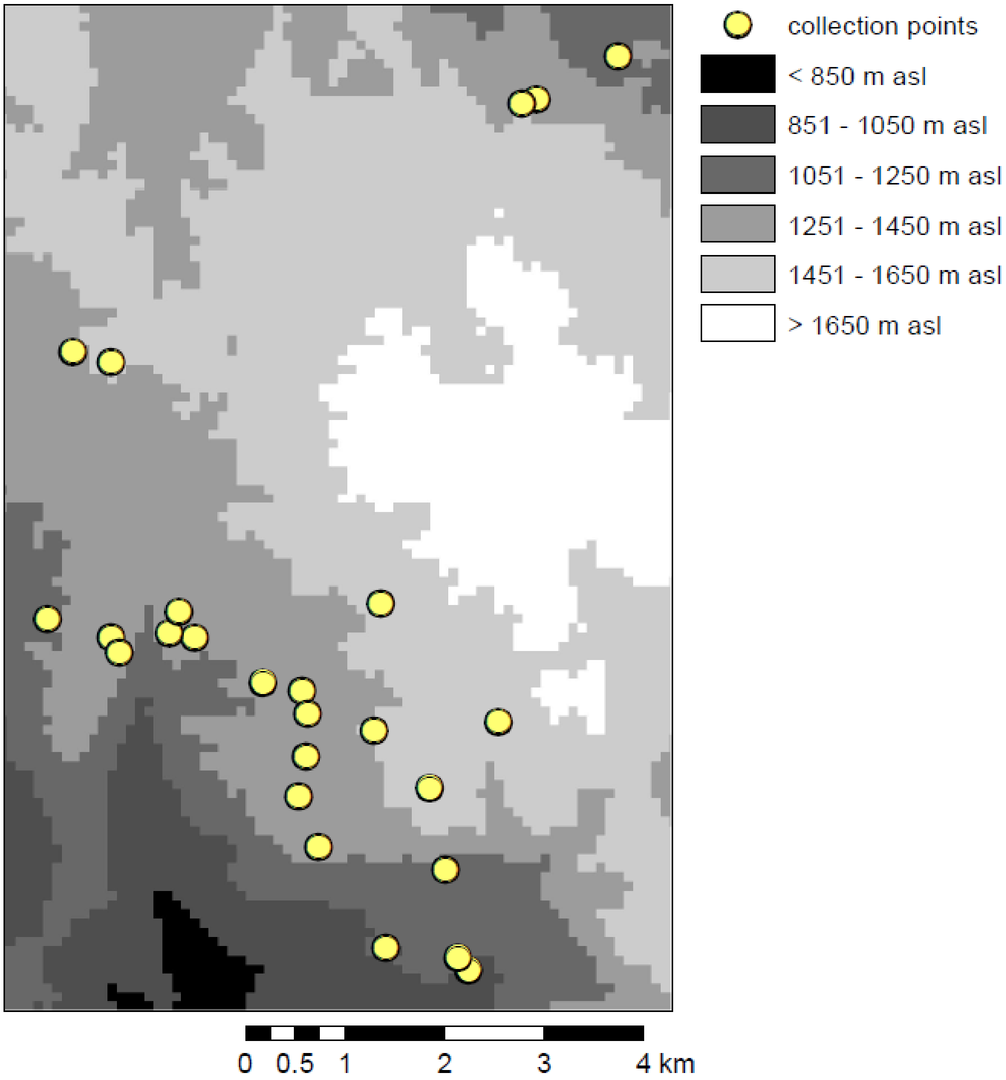

Fig. 2 Map of elevation bands with locations of sampled colonies. Elevation bands (friction values) are < 850 m (10), 851-1050 m (30), 1051-1250 m (50), 1251-1450 m (70), 1451-1650 m (90), and > 1650 m (100). Friction values are estimates of elevation resistance with 100 being a complete barrier to dispersal.

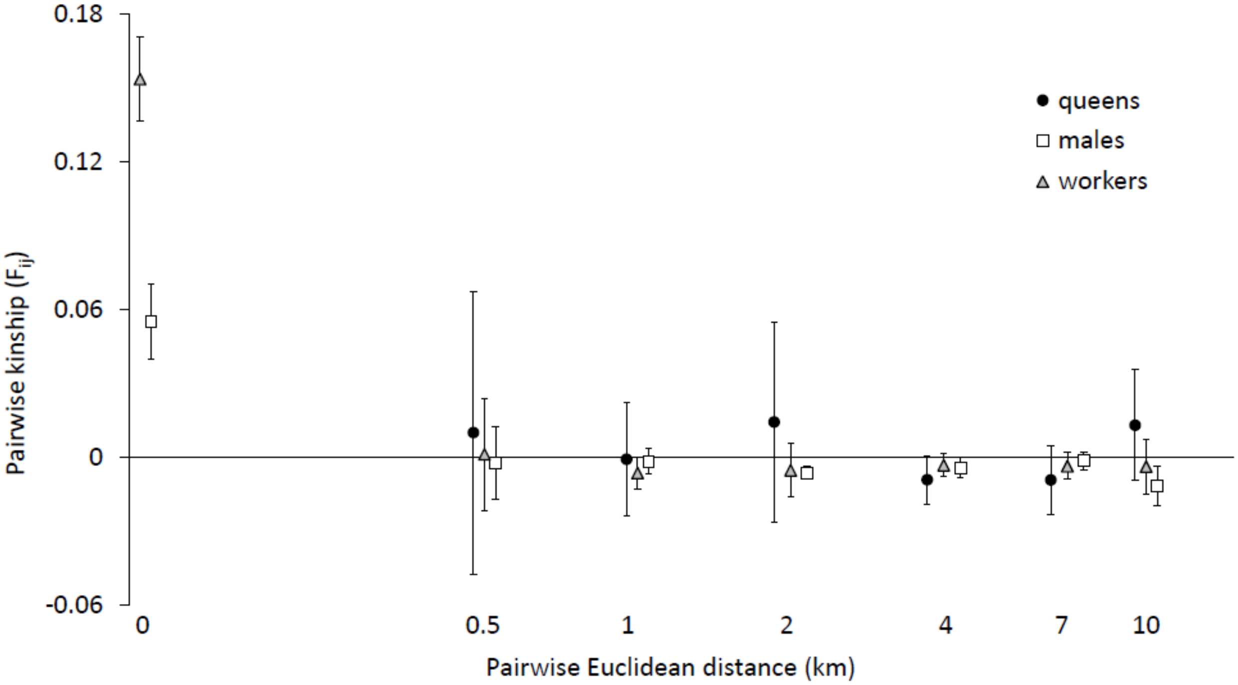

Fig. 3 There was no spatial genetic structure (pairwise kinship coefficient, F_{ij}) for queens, males, and workers in a population of *Eciton burchellii parvispinum* in Monteverde, Costa Rica. Distance intervals are 0 (within colony), 0-0.5 km (not including within colony), 0.5-1 km, 1-2 km, 2-4 km, 4-7 km, and 7-10 km, graphed on the natural logarithm of Euclidean distance. Error bars represent +/- 2SE. Values offset to facilitate visualization.

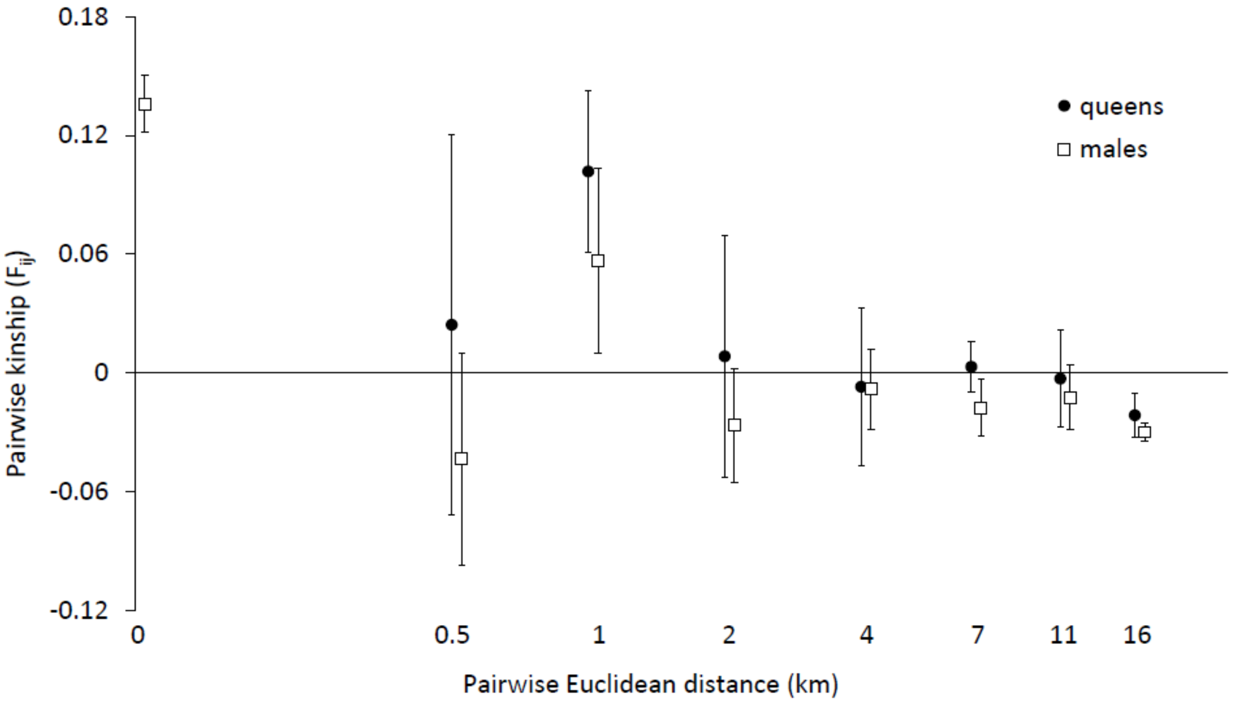

Fig. 4 Spatial genetic structure (pairwise kinship coefficient, F_{ij}) for queens and males in a population of *Eciton burchellii foreli* in San Lorenzo, Panama. Distance intervals are 0 (within colony), 0-0.5 km (not including within colony), 0.5-1 km, 1-2 km, 2-4 km, 4-7 km, 7-11 km, and 11-16 km, graphed on the natural logarithm of Euclidean distance. Error bars represent +/- 2SE. Values offset to facilitate visualization.

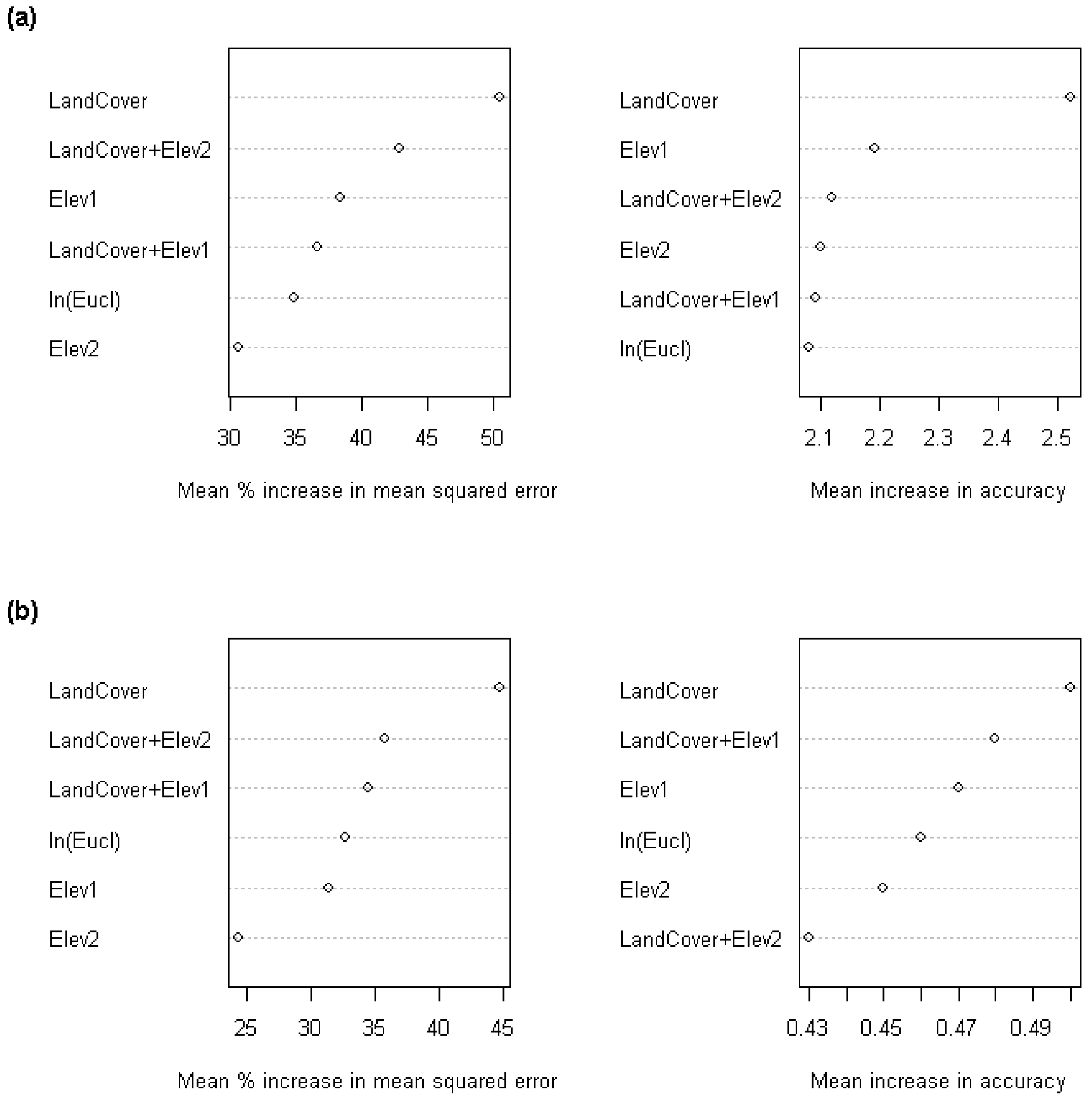

Fig. 5 Ranking of variable importance in random forests (RF) analyses for predicting (a) the pairwise relationship coefficient (r_{ij}) and (b) the pairwise kinship coefficient (F_{ij}). Mean percent increase in mean squared error (MSE) is the normalized difference in MSE for predicting the out-of-bag (OOB) data when the variable is included in the model as observed versus that when it is randomly permuted, averaged over all trees. Random permutations of an important variable will lead to large relative increases in MSE. Mean increase in accuracy is the reduction in node impurities when splitting on the variable, as measured by residual sum of squares, averaged over all trees.

Supporting information

Table S1 Annealing temperatures (T_a) for seven primers, number of alleles (N_a), range of allele sizes, and observed (H_o) and expected (H_e) heterozygosities at each locus. We amplified fragments in 10 µl total volume polymerase chain reactions (PCR): 5 µl of QIAGEN multiplex PCR master mix (2x), 2 µl of 0.5 ng DNA, and 0.2 µM labeled primers (QIAGEN Inc., Valencia, CA, USA). PCR cycles comprising an initial denaturation step of 95 ˚C for 15 min, denaturation step of 94 ˚C for 30 sec, annealing step of 1 min 30 sec with T_A ranging from 50-63 ˚C depending on the primer, extension step of 72 ˚C for 1 min, number of cycles ranging from 30-35, and a final extension step of 72 ˚C for 30 min were used (QIAGEN Inc.).

Primer	**T_a (°C)**	**N_a**	**Size range (bp)**	**H_o**	**H_e**
*Eb04**	50	11	77 - 121	0.78	0.71
Eb10†	57	13	148 - 206	0.77	0.79
Eb21‡	57 - 61	6	124 - 163	0.39	0.41
*Eb24**	57	26	130 - 233	0.94	0.96
*Eb25**	59 - 63	12	137 - 173	0.56	0.53
Eb42‡	57 - 61	12	104 - 153	0.78	0.78
Eb51†	57	18	89 - 154	0.93	0.89

* - amplified separately

†,‡ - amplified together (multiplexed)

Table S2 Alternative models of landscape resistance predicting pairwise queen relatedness. Statistical significance was stable to changes in friction values and resistance distances, except in two cases: significance lost when the land cover class urban/bare earth and when the highest elevations (> 1650 m asl) were not considered complete barriers to dispersal (models 2.g and 3.f). Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values represent significant relationships (B-Y FDR adjusted alpha = 0.0161). PF = primary forest, SF = secondary forest, EH = edge habitat, AG/P = agriculture/pasture, U/BE = urban/bare earth.

		Pairwise relationship (r_{ij})			**Pairwise kinship (F_{ij})**		
Model	**Change (friction values)**	***b***	**r^2**	***P***	***b***	**r^2**	***P***
2.b	natural logarithm of original resistance distances	**-0.0709**	**0.0444**	**0.007**	-0.0160	0.0105	0.075
2.c	original resistance distances squared	**-0.00002**	**0.0424**	**0.004**	-0.000004	0.0072	0.106
2.d	more similarity in friction values for more heavily vegetated land cover classes (PF, SF, & EH): PF(20), SF(30), EH(40), AG/P(70), U/BE(100)	**-0.0020**	**0.0422**	**0.007**	-0.0005	0.0105	0.062
2.e	friction values based on Pérez-Espona *et al.* 2012a: PF(1), SF(2), EH(10), AG/P(50), U/BE(100)	**-0.0038**	**0.0428**	**0.005**	-0.0007	0.0073	0.129
2.f	alternative friction values based on combination of original friction values and those based on Pérez-Espona *et al.* 2012a: PF(10), SF(20), EH(40), AG/P(80), U/BE(100)	**-0.0019**	**0.0454**	**0.004**	-0.0004	0.0091	0.086
2.g†	urban/bare earth not complete barrier to dispersal: PF(10), SF(30), EH(50), AG/P(70), U/BE(90)	-0.0039	0.0306	0.018	-0.0006	0.0035	0.292
3.b	natural logarithm of original resistance distances	**0.1188**	**0.0226**	**0.016**	0.0182	0.0025	0.377
3.c	original resistance distances squared	**0.00002**	**0.0252**	**0.012**	0.000003	0.0014	0.522
3.d	continuous linear elevation friction values with 100m elevation bands and above 1700m as complete barrier to dispersal: < 800m(1), 801-900m(10), 901-1000m(20), 1001-1100m(30), 1101-1200m(40), 1201-1300m(50), 1301-1400m(60), 1401-1500m(70), 1501-1600m(80), 1601-1700m(90), >1700m(100)	**0.0032**	**0.0273**	**0.011**	0.0005	0.0028	0.341

3.e	alternative linear friction values with original 200m elevation bands: <850m(1), 851-1050m(20), 1051-1250m(40), 1251-1450m(60), 1451-1650m(80), >1650m(100)	**0.0023**	**0.0285**	**0.005**	0.0003	0.0024	0.368
3.f†	above 1650m not complete barrier to dispersal: <850m(10), 851-1050m(20), 1051-1250m(30), 1251-1450m(50), 1451-1650m(70), >1650m(90)	0.0037	0.0197	0.040	0.0007	0.0031	0.310
4.b	natural logarithm of original resistance distances	-0.0422	0.0035	0.387	-0.0185	0.0032	0.312
4.c	original resistance distances squared	-0.000003	0.0070	0.256	-0.000001	0.0053	0.186
4.d	multivariate (additive) combination of friction values from 2.f and 3.e	-0.0007	0.0073	0.253	-0.0003	0.0047	0.232
4.e	natural logarithm of resistance distances from 4.d	-0.0446	0.0046	0.337	-0.0166	0.0030	0.338
4.f	resistance distances from 4.d squared	-0.000004	0.0102	0.174	-0.000002	0.0060	0.157
4.g	multivariate (additive) combination of friction values from 2.g and 3	0.0014	0.0128	0.077	0.0001	0.0005	0.738
5.b	natural logarithm of original resistance distances	0.0287	0.0034	0.365	0.0026	0.0001	0.891
5.c	original resistance distances squared	0.0001	0.0099	0.131	0.00001	0.0017	0.492
5.d	square root of original resistance distances	0.0176	0.0051	0.262	0.0022	0.0004	0.755
6.b	natural logarithm of original resistance distances	-0.0533	0.0158	0.082	-0.0146	0.0055	0.185
6.c	original resistance distances squared	-0.00001	0.0276	0.023	-0.000002	0.0058	0.169
6.d	square root of original resistance distances	-0.0175	0.0197	0.063	-0.0044	0.0059	0.175
6.e	multivariate (additive) combination of friction values from 2.g and 5	-0.0005	0.0010	0.620	-0.0001	0.0001	0.801

† - represents a loss of statistical significance compared to original model

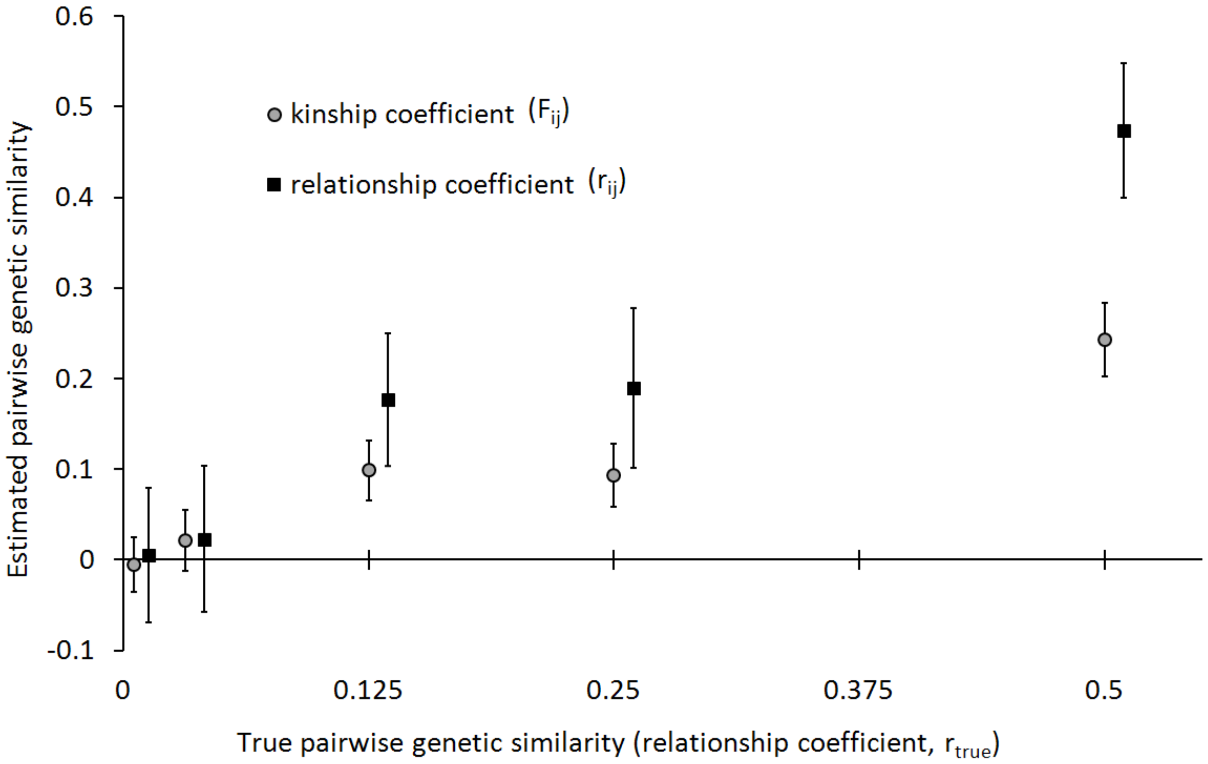

Fig. S1 Plot of estimates of genetic similarity of simulated dyads of known relationships. The two measures of genetic similarity were able to distinguish among levels of simulated relationships (known relationships represented as relationship coefficient, r_{true}). Simulated genotypes were based on allele frequencies of seven loci from the Monteverde population. Estimates of pairwise kinship coefficient (F_{ij}) and pairwise relationship coefficient (r_{ij}) were calculated by the program SPAGeDi. Error bars represent a 95% confidence interval around the mean. Values offset to facilitate visualization.

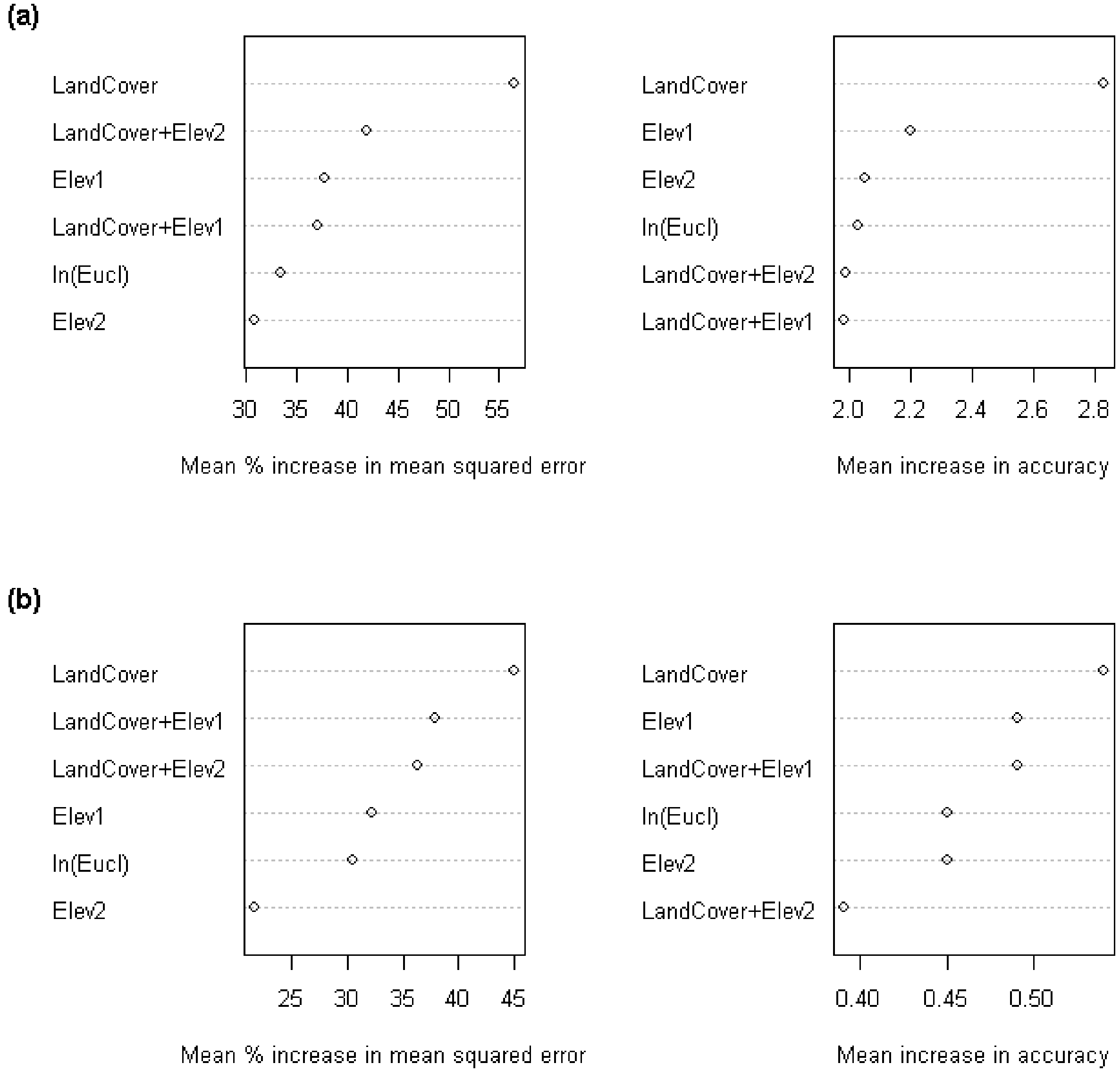

Fig. S2 Ranking of variable importance in random forests (RF) analyses for predicting (a) the pairwise relationship coefficient (r_{ij}) and (b) the pairwise kinship coefficient (F_{ij}) with random variable subsets (m_{try}) of size 4. Mean percent increase in mean squared error (MSE) is the normalized difference in MSE for predicting the out-of-bag (OOB) data when the variable is included in the model as observed versus that when it is randomly permuted, averaged over all trees. (Random permutations of an important variable will lead to large relative increases in MSE). Mean increase in accuracy is the reduction in node impurities when splitting on the variable, as measured by residual sum of squares, averaged over all trees.

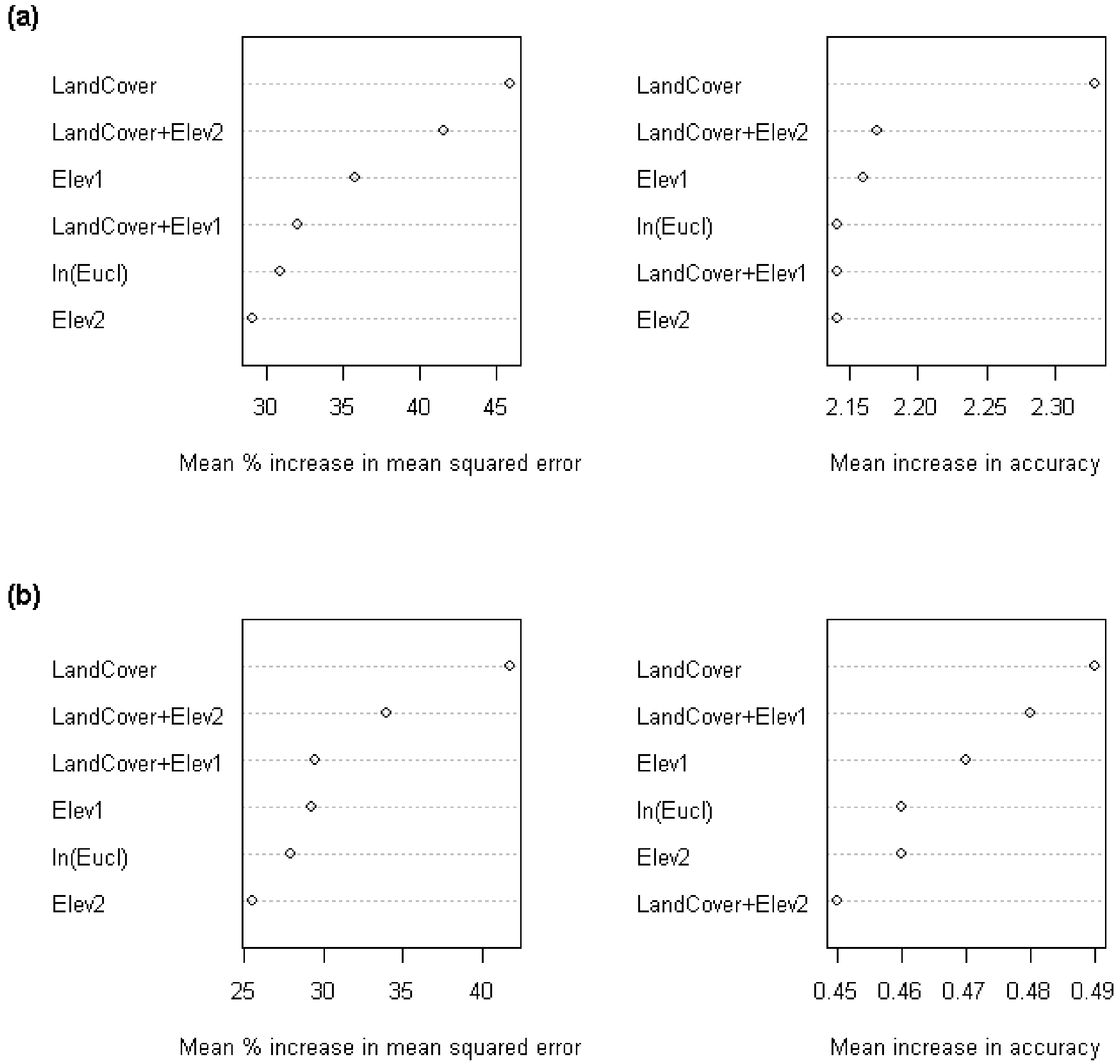

Fig. S3 Ranking of variable importance in random forests (RF) analyses for predicting (a) the pairwise relationship coefficient (r_{ij}) and (b) the pairwise kinship coefficient (F_{ij}) with random variable subsets (m_{try}) of size 1. Mean percent increase in mean squared error (MSE) is the normalized difference in MSE for predicting the out-of-bag (OOB) data when the variable is included in the model as observed versus that when it is randomly permuted, averaged over all trees. (Random permutations of an important variable will lead to large relative increases in MSE). Mean increase in accuracy is the reduction in node impurities when splitting on the variable, as measured by residual sum of squares, averaged over all trees.

Chapter 3: Dispersal distance in the army ant *Eciton burchellii*

Abstract

Dispersal distance, important for maintenance of genetic diversity, is affected by ecological factors, life history traits, and behavior of animals. Restricted dispersal can lead to spatial genetic structure (SGS), high local genetic similarity, or inbreeding depression and local extinction. Army ants have wingless queens and colonies reproduce through fission, yet males are winged and may be responsible for the majority of gene flow. However, colonies of the army ant *Eciton burchellii* regularly move due to obligate nomadism and summed migrations over the lifetime of the queen have potential to contribute to gene flow in continuous populations. To determine whether army ant colony emigrations contribute to gene flow we examined two *E. burchellii* cohorts in Monteverde, Costa Rica for direct dispersal events. We also compared individual-based relatedness estimates across sexes for estimates of indirect dispersal distance. We also re-evaluated the effects of landscape features on gene flow. Though we did not detect any parent-offspring relationships (direct dispersal events), a colony was re-sampled 3 years later. Significant SGS was present in males but not queens. Queens were significantly unrelated to queens in the same area three years previous. This suggests that colonies migrate at least 500 m over their lifetimes. Gene dispersal in army ants seems to be greater than previously thought and colony emigrations over the lifetime of the queen may contribute to gene flow. The effect of forest cover on queen dispersal was present in the first sampling year and when both years were combined but not in the second year. Thus temporal replication, in addition to spatial replication, is important for evaluating the effects of landscape features on patterns of dispersal. Habitat connectivity should be maintained to enable gene flow, supporting populations of this keystone species and the biodiversity which it generates.

Introduction

Dispersal has important effects at all spatial scales and levels of biological organization (Greenwood 1980; Johnson & Gaines 1990; Broquet and Petit 2009; Whitmee & Orme 2013). Animal movements and subsequent gene dispersal affect the evolution of populations and ecosystems, including responses to fragmentation, species invasions, and climate change (Nathan *et al.* 2008). Within populations, viscosity affects kinship and therefore has the potential to influence conflict and cooperation (Greenwood 1980; Lawson Handley & Perrin 2007; Clutton-Brock & Lukas 2012). Among populations, dispersal increases gene flow and tips the balance between homogenization on the one hand and possible outcomes speciation and local extinction on the other (Saccheri *et al.* 1998; Turelli *et al.* 2001). Gene flow is accomplished via migrants joining new populations and reproducing successfully (Greenwood 1980). Depending on the life history of a species, short- and long-distance animal movements can contribute to dispersal and subsequent gene flow.

Short- and long-distance animal movements affected by ecological forces, such as wind and water currents (Fonseca & Hart 1996; Lorch *et al.* 2005; Mandel *et al.* 2008; Shanks 2009), temperature (Nieminen 1996; Biuw *et al.* 2007), resource availability and distribution (Lurz *et al.* 1997; Broquet *et al.* 2006; Fryxell *et al.* 2008; Wittemyer *et al.* 2008; Messier *et al.* 2012), mortality risk (Revilla & Wiegand 2008; Wittemyer *et al.* 2008; Johnson *et al*. 2009), population density (Fonseca & Hart 1996; Lorch *et al.* 2005; Matthysen 2005; Busch *et al.* 2009; Molina-Morales *et al.* 2012), kinship (Lena *et al.* 1998; Lena *et al.* 2000; Armitage *et al.* 2011), and habitat fragmentation (Saccheri *et al.* 1998) and matrix heterogeneity (Revilla & Wiegand 2008). Individual dispersal distances are also dependent on life history traits such as mating system (Greenwood 1980; Nussey *et al.* 2005) mode of reproduction (Pamilo *et al.* 1997; Peeters & Ito 2001; Nichols *et al.* 2012), and body size within- and across- species (Harris & Trewhella 1988; Nieminen 1996; Molina-Morales *et al.* 2012; Whitmee & Orme 2013). Home range area, geographic range size, phylogeny, fecundity, and ecological specialization were also found to be important determinants of dispersal distances across species (Nieminen 1996; Steffan-Dewenter & Tscharntke 2000; Stevens *et al.* 2012; Whitmee & Orme 2013). The decision of when and where to disperse is affected by behaviors such as previous experience (Forero *et al.* 1999; Serrano *et al.* 2001) and social rank (Wittemyer *et al.* 2008). The timing of reproduction and choice of spatial location affect dispersal distances as well (Shanks 2009). Behavior can also restrict gene flow through sexual selection (West-Eberhard 1983; Doherty *et al.* 2003) and diet (Pilot *et al.* 2006; Pilot *et al.* 2012). Restricted gene flow can lead to speciation (Turelli *et al.* 2001) or inbreeding depression and local extinction (Saccheri *et al.* 1998). With limited dispersal, genetic similarity will be high among neighboring individuals relative to distant individuals, a phenomenon known as spatial genetic structure (SGS) (Vekemans & Hardy 2004). To avoid breeding with related individuals (or to increase access to mates or resources), some species exhibit sex-biased dispersal where one sex disperses out of the natal area and thus may be responsible for the majority of gene flow among populations (Greenwood 1980; Lawson Handley & Perrin 2007; Clutton-Brock & Lukas 2012).

Sex-biased dispersal has implications for conflict and cooperation within and between sexes (Greenwood 1980; Lawson Handley & Perrin 2007; Clutton-Brock & Lukas 2012), including number of breeders, reproductive skew, and worker policing within social insect colonies (Pamilo *et al.* 1997). Sex-biased dispersal in ants often develops a consequence of dependent colony founding, in which a daughter queen disperses on foot with a portion of the worker force while males independently disperse on the wing (Pamilo *et al.* 1997; Peeters & Ito 2001; Sundström *et al.* 2005). Army ants are dependent colony founders (Franks 1985; Kronauer 2009) but males are winged; the combination of these traits implies male-biased dispersal (Seppä & Pamilo 1995; Pamilo *et al.* 1997; Sundström *et al.* 2005). However, *E. burchellii* army ants are obligately and regularly nomadic (Schneirla 1971; Gotwald 1995; Kronauer 2009), and cumulative short term movements (500 m every 5 weeks: Franks & Fletcher

1983; Willson *et al.* 2011) before reproduction (3 years: Franks 1985) have the potential to facilitate gene flow.

Recent genetic analyses of army ants have uncovered conflicting evidence regarding sex-biased dispersal. While mitochondrial DNA has been found to have more spatial structure than nuclear microsatellites (Berghoff *et al.* 2008; Kronauer *et al.* 2010; Pérez-Espona *et al.* 2012), mtDNA has a much slower mutation rate and may lack the spatial and temporal resolution to detect fine-scale and contemporary gene flow (Foitzik *et al.* 2009; Storfer *et al.* 2010; Wang 2010, 2011). Spatial autocorrelation of individual-based relatedness estimates based on nuclear loci has detected SGS in one population of army ants but not a second (Soare *et al.* in press). To determine whether collective movements over the lifetime of the colony contribute to gene flow, we sampled two generations of the army ant *Eciton burchellii* and compared direct and indirect measures of dispersal. We also assessed whether spatial genetic structure and the effect of landscape features on dispersal remain stable over time. Though the importance of spatial replication in landscape genetics has been recognized (Segelbacher *et al.* 2010; Angelone *et al.* 2011; Short Bull *et al.* 2011), temporal replication allows re-evaluation of factors affecting current evolutionary processes.

Methods

Study site and sample collection

In July and August 2009, we located *Eciton burchellii* colonies in montane forest around Monteverde, Costa Rica through systematic trail walks and opportunistic encounters (Vidal-Riggs & Chaves-Campos 2008; Kumar & O'Donnell 2009; Soare *et al.* 2011, in press). We thoroughly sampled areas that were sampled for a previous study (Soare *et al*. in press) to maximize our ability to detect direct dispersal events. Because a sampling distance of at least 10 times gene dispersal distance (σ) is necessary to obtain reliable estimates of gene dispersal (Vekemans & Hardy 2004; Watts *et al.* 2007), and male dispersal distance was estimated at 1 km (Jaffé *et al.* 2009), we sampled over distances of about 10 km E-W and 10 km N-S. For all collections, we placed 25-40 worker ants of all castes directly into 95% EtOH, and recorded latitude, longitude, and elevation to the nearest 10 m with a handheld GPS unit. Both sides of the continental divide were sampled (Pacific slope: 1000 – 1650 m asl, Atlantic slope: 1000 – 1400 m asl). All samples were subspecies *E. b. parvispinum* Forel (1899).

Genetic analyses

After extracting DNA with a Chelex protocol (Walsh 1991), we amplified eight polymorphic microsatellite loci: *Eb04, Eb10, Eb14, Eb21, Eb24, Eb25, Eb42, Eb51* (Denny *et al.* 2004; Kronauer *et al.* 2006; Berghoff *et al.* 2008; Jaffé *et al.* 2009; Pérez-Espona *et al.* 2012; Soare *et al.* in press). One locus (*Eb14*) was dropped due to poor amplifications. (See Soare *et al.* in press for details on PCR conditions.) Fragment analyses were performed on an ABI 3730 sequencer, and scored in GeneMapper 4.0 software (Applied Biosystems, Carlsbad, CA, USA). All allele scores were checked manually. A subset of alleles was re-scored and the error rate was 0.1% (DeWoody *et al.* 2006; Selkoe & Toonen 2006; Guichoux *et al.* 2011).

Statistical analyses

We reconstructed queen and male genotypes in COLONY 2.0 (Jones & Wang 2010; Soare *et al.* in press, 2013). To confirm reliability of our results, we performed multiple reconstructions with different random number seeds. Cohorts were compared for differentiation by calculating Hedrick's G'_{ST} and Jost's D_{est} in SMOGD (Hedrick 2005; Jost 2008; Crawford 2010; Balkenhol *et al.* 2013). Queens were tested for inbreeding by calculating F_{Iq} in SPAGeDi 1.3 (Hardy & Vekemans 2002) and by calculating linkage disequilibrium in FSTAT 2.9.3.2 with 420 permutations (nominal $P = 0.0024$) (Goudet 2002). Queen effective mating frequency (m_e: Nielsen *et al.* 2003) was compared across cohorts and to data from Barro Colorado Island, Panama (Kronauer *et al.* 2006); Henri Pittier National Park, Venezuela

(Kronauer & Boomsma 2007); and Chiapas, Mexico (Jaffé *et al.* 2009) with Wilcoxon rank sum tests in the stats package in R 2.14.1 (R Development Core Team 2011).

To determine if any queens or males sampled in 2009 were offspring of the 2006 cohort, we performed parentage analyses in COLONY 1.3 (males) and COLONY 2.0 (queens) (Wang 2004; Jones & Wang 2010). To evaluate sex patterns of dispersal, we regressed the pairwise kinship coefficient (F_{ij}: Loiselle *et al.* 1995) on the natural logarithm of Euclidean distance and evaluated the slope of the regression line with 10000 permutations in SPAGeDi 1.3 & 1.4 (Hardy & Vekemans 2002; Hardy *et al.* 2008). To estimate gene dispersal (σ), we used an estimate of effective population density based on averaging army ant population density estimates from five sites (Barro Colorado Island, Panama: Franks 1982; Corcovado National Park, Costa Rica: Swartz 1997; La Selva Biological Station, Costa Rica: Vidal-Riggs & Chaves-Campos 2008; Chiapas, Mexico: Jaffé *et al.* 2009; Cocha Cashu Biological Station, Peru: Willson *et al.* 2011), and calculating the effective population size (Wright 1933; Jaffé *et al.* 2009) with the average effective mating frequency from three sites (Barro Colorado Island, Panama: Kronauer *et al.* 2006; Henri Pittier National Park, Venezuela: Kronauer & Boomsma 2007; Chiapas, Mexico: Jaffé *et al.* 2009). As one-third of colonies reproduce annually (Franks 1985; Kronauer 2009), we divided the effective population size by three to obtain an estimate of effective population density. This conforms to expectations that effective population density is one-tenth to one-half of population census size (Vekemans & Hardy 2004). To examine temporal patterns of relatedness, we regressed pairwise kinship (F_{ij}) across cohorts on the natural logarithm of Euclidean distance in SPAGeDi. We compared 2009 queens to 2006 queens and 2009 males to 2006 queens as males are haploid and have no father (Hölldobler & Wilson 1990).

Spatial analyses

To evaluate the effects of landscape on spatial genetic structure on queens, we followed a landscape genetics approach and treated samples as being derived from a continuous population and carried out all analyses at the level of the individual (Manel *et al.* 2003; Segelbacher *et al.* 2010; Storfer *et al.* 2010). We considered army ant dispersal in Monteverde to be affected by landscape variables in six basic ways: isolation by Euclidean distance (Model 1), isolation by land cover resistance (Model 2), isolation by elevation resistance (Model 3), isolation by combined land cover and elevation resistance (Model 4), isolation by elevation band resistance (increasing friction to disperse out of own elevation band, Model 5), and isolation by combined land cover and elevation band resistance (Model 6). To compare these models of dispersal, we constructed friction maps based on the five resistance models and calculated pairwise resistance distances among collection locations with the software CIRCUITSCAPE 3.5.4 (McRae 2006; McRae & Beier 2007; McRae *et al.* 2008). Resistance distances are based on circuit theory and simultaneously consider all possible pathways between nodes, with multiple or wider connecting corridors allowing greater gene flow (McRae 2006; McRae & Beier 2007; McRae *et al.* 2008).

For models 2, 4, & 6, we acquired multispectral satellite imagery (Landsat 7 ETM+, 30 m resolution) from the United States Geological Survey (USGS: earthexplorer.usgs.gov) and screened for images that had minimal cloud coverage over the Monteverde area (path/row: 15/53). We analyzed one image captured in wet season (6/15/2001) and one image from the following dry season (3/30/2002). For models 3 - 6, we downloaded a digital elevation model of the Monteverde area (SRTM, 90 m resolution) from the USGS website. All spatial analyses were performed in ArcGIS 10.0 (ESRI Inc., Redlands, CA, USA). Both Landsat images were pan-sharpened to 15 m resolution (ArcGIS 10.0 Resource Center, ESRI, 2011). We ran an Iso Cluster unsupervised classification on spectral bands 1 – 6 (ArcGIS 10.0 Resource Center, ESRI, 2011), and identified five final land cover classes (primary forest, secondary forest, edge, agriculture/pasture, urban/bare earth) based on satellite imagery, ecological data taken during sample collection and for previous studies (Kumar and O’Donnell 2009), and land use maps of the Monteverde area (Nadkarni & Wheelwright 2000). Classified land cover rasters were combined into one output raster by prioritizing more disturbed land cover classes (higher friction values) when input values disagreed and shrinking areas of missing data (ArcGIS 10.0 Resource Center, ESRI, 2011). We created friction maps by assigning values to land cover classes and elevation bands. Friction values are arbitrary and range from 0 to 100, but increasing friction values reflect greater habitat resistance to dispersal (McRae & Beier 2007; Goulson *et al.* 2011). Our six *a priori* models, one isolation by distance (IBD) and five isolation by resistance (IBR), were:

Model 1 – Isolation by distance (IBD). Euclidean distance was calculated as the straight line distance between collection points in a two-dimensional landscape, and the natural logarithm of this distance was used (as described for tests of sex-biased dispersal, above).

Model 2 – Land cover. *E. burchellii* rarely crosses open areas in lowland rainforest (Meisel 2006), but forages more frequently in open areas as elevation increases, possibly due to less drastic temperature differences between open and forested areas at high elevations (Kumar & O'Donnell 2009). Deforestation was found to inhibit dispersal of *E. burchellii (*Pérez-Espona *et al.* 2012; Soare *et al.* in press). Higher friction values were assigned to land classes of decreasing forest cover: primary forest = 10, secondary forest = 30, edge = 50, agriculture/pasture = 70, and urban/bare earth = 100, with 100 as a complete barrier to dispersal (Fig. 1). Grids consisted of 1041 X 1346 cells (15 m cell size).

Model 3 – Elevation 1. Army ant raids were found less frequently as elevation increased in Monteverde, Costa Rica (Kumar & O'Donnell 2009). The highest recorded observation of *E. burchellii* in the Monteverde area is 1622 m asl on the Pacific slope (Soare *et al.* 2011), though *E. burchellii* has been collected at 1670 m asl in the Talamanca mountain range in southern Costa Rica (J. T. Longino *pers comm.*; antweb.org). Higher friction values were assigned to elevation bands of equal altitudinal width of increasing elevation: < 850 m = 10, 851 – 1050 m = 30, 1051 – 1250 m = 50, 1251 – 1450 m = 70, 1451 – 1650 m = 90, and > 1650 m = 100, with 100 as a complete barrier to dispersal (Fig. 2). Grids consisted of 173 X 200 cells (90 m cell size).

Model 4 – Land cover + Elevation 1. Friction maps from Models 2 and 3 were combined such that each cell of the resulting raster took on the maximum value of the input cells. This combined raster measures the additive effect of both land cover and elevation, and is equivalent to a multivariate approach (Spear *et al.* 2010). A friction value of 100 represents a complete barrier to dispersal. Grids consisted of 854 X 721 cells (15 m cell size).

Model 5 – Elevation 2. Though army ant encounter rate declines with elevation (Kumar & O'Donnell 2009), the Monteverde area consists of several Holdridge life zones (loosely correlated with elevation) (Holdridge 1967; Haber 2000), and colonies may be adapted to local environmental conditions. Colonies were assigned to elevation bands of at least 80 m in altitudinal width in an attempt to equalize colony samples sizes among bands: 950 – 1220 m (n = 5), 1221 – 1357 m (n = 5), 1358 – 1438 m (n = 11), 1439 – 1650 m (n = 4). Friction values were assigned such that a focal queen dispersing within her elevation band = 10, dispersing to a neighboring elevation band = 30, dispersing to a non-neighbor elevation band = 50, dispersing to a distant elevation band (separated by two or more bands) = 70, and > 1650 m = 100 (complete barrier to dispersal). Grids consisted of 173 X 200 cells (90 m cell size).

Model 6 – Land cover + Elevation 2. Friction maps from Models 2 and 5 were combined such that each cell of the resulting raster took on the maximum value of the input cells. This combined raster measures the additive effect of both land cover and elevation band, and is equivalent to a multivariate approach (Spear *et al.* 2010). A friction value of 100 represents a complete barrier to dispersal. Grids consisted of 854 X 720 cells (15 m cell size).

We regressed the pairwise relationship coefficient (r_{ij}: Queller & Goodnight 1989) among queens on resistance distances for each model in SPAGeDi 1.3 (Hardy & Vekemans 2002; Soare *et al.* in press). Coefficients of determination (r^2) were compared among models to determine which resistance distance accounted for the most variation in pairwise relatedness among queens (highest r^2 values) (McRae

2006). To account for testing six models of dispersal, the critical alpha level was adjusted with Benjamini and Yekutieli false discovery rate procedure (B-Y FDR: Benjamini & Yekutieli 2001; Narum 2006).

Results

Quality of genetic data

After initially genotyped 15 workers from each of 17 collections, three workers were removed for having genotypes inconsistent with the majority matriline. These workers were likely daughters of previously replaced queens. The remaining workers (n = 252, 13-15 per colony) were genotyped at five or more loci (233 workers had full genotypes at all loci).

Population descriptive statistics

16 queen and 174 male genotypes were reconstructed from the 17 collections; thus one colony was sampled twice. The two collections that had the same reconstructed queen genotype also shared 3 patrilines. The newly sampled male genotypes were considered to have the spatial coordinates of this first collection location in all spatial analyses. Another colony had the same reconstructed queen genotype and shared 6 patrilines as a collection from 2006; thus we had re-sampled the same colony three years later. Because queens do not mate repeatedly throughout their lifetimes (Kronauer *et al.* 2006; Kronauer & Boomsma 2007), the three newly discovered patrilines were considered as belonging to the 2006 cohort and were assigned the spatial coordinates of the original collection location in all analyses. Repeat COLONY runs with different random number seeds returned the same results for the 2009 worker dataset and a combined worker dataset of both sampling years.

The 15 newly sampled colonies had an effective mating frequency of 15.4 +/- 2.3 SE. This did not significantly differ from the previously sampled cohort (Wilcoxon rank sum test W = 119, *P* = 0.057). When both sampling years were combined, Monteverde queens had an effective mating frequency of

12.4 +/- 1.3 SE, which did not differ from mating frequencies in Barro Colorado Island, Panama; Henri Pittier National Park, Venezuela; and Chiapas, Mexico (Wilcoxon rank sum test W = 811, $P = 0.642$). Allele frequencies were not significantly different across the two sampled years (Jost's $D_{est} = 0.000066$ +/- 0.000105 SE; Hedrick's $G'_{ST} = 0.000064$ +/- 0.000130 SE). When both sampling years were combined, the queen inbreeding coefficient, F_{Iq}, not significantly different from zero (-0.054 +/- 0.036 SE, $P = 0.053$). For each locus, F_{Iq} was not significantly different from zero (each $P > 0.00714$, the nominal P after Bonferroni adjustment for multiple comparisons). There was no significant linkage among any pairs of loci (each $P > 0.0024$, the nominal P after Bonferroni adjustment for multiple comparisons). Males mated to the same queen were significantly related ($F_{mm} = 0.0586$ +/- 0.005, $P < 0.0001$).

Parentage analysis

No queens or males sampled in 2006 were identified as probable parents of 2009 queens in COLONY 2.0 (each $P < 0.394$ where a high P represents high certainty). No queens sampled in 2006 were identified as mothers of 2009 males in COLONY 1.3. COLONY results were robust to repeat runs with different random number seeds.

Spatial genetic structure across sexes

There was no significant spatial genetic structure in queens in 2009 ($b = -0.0136$, $r^2 = 0.0195$, $P = 0.224$) or over both years combined ($b = -0.0061$, $r^2 = 0.0031$, $P = 0.218$, Table 1). Although not found in 2006, males exhibited significant spatial genetic structuring in 2009 ($b = -0.0069$, $r^2 = 0.0012$, $P = 0.019$) and over both years combined ($b = -0.0030$, $r^2 = 0.0002$, $P = 0.012$; Table 1).

To estimate gene dispersal (σ), we used an estimate of effective population density based on data from other sites and an estimate based on data from Monteverde. The average population density across all army ant populations studied to date was 5.48 colonies per km^2. Using an effective mating frequency averaged across army ant populations, the effective population size of army ants is 11.81 individuals per km^2 with an effective population density of 3.94 individuals per km^2 (in reproductive phase). We also used the re-sampled colony to obtain a population density estimate for the Monteverde area. With an estimated mortality rate of 50% after three years (Franks 1985), the Lincoln-Petersen mark-recapture method yielded an estimate of 2.66 colonies per km^2, which corresponds to an effective population size of 5.76 individuals per km^2 and an effective population density is 1.92 individuals per km^2. Although we found significant spatial genetic structure in males over both years combined, we were unable to obtain a gene dispersal estimate (σ) for males based on pairwise kinship (F_{ij}) because the iterative procedure regressing kinship on distance did not converge for either effective population density estimate.

The regression of pairwise kinship between queen cohorts on pairwise Euclidean distance was not significant ($b = 0.0056$, $r^2 = 0.0051$, $P = 0.234$). However, 2009 queens were significantly unrelated to 2006 queens within 0.5 km ($F_{ij} = -0.039$ +/- 0.015 SE, $P = 0.044$, Figure 2). There was no relationship between kinship between 2009 males and 2006 queens and Euclidean distance ($b = 0.0001$, $r^2 < 0.0001$, $P = 0.957$).

Landscape genetics

The significant isolation by landscape resistance for 2006 queens was not replicated in 2009 queens ($b = 0.0024$, $r^2 = 0.0106$, $P = 0.546$; Table 2). However, when both sampling years were combined, resistance distances based on land cover significant predicted pairwise relatedness among queens ($b = -0.0031$, $r^2 = 0.0240$, $P = 0.013$).

Discussion

We detected spatial genetic structure in males but not in queens. Queens were unrelated to queens from the previous cohort in same area (within 0.5 km). No likely parents of 2009 cohort were detected, limiting our ability to obtain a direct dispersal estimate. We sampled a colony 3 years later in the same area, confirming previous lifespan estimates (Rettenmeyer 1963; Franks 1985). Cohorts did not display the same pattern of isolation by landscape resistance, pointing to a need for temporal replication in landscape genetics studies.

Male pairwise relatedness declined with Euclidean distance in the 2009 cohort and when both cohorts were combined, suggesting a maximum flight distance for males that is less than our spatial extent of 10 km. However, we were unable to obtain an indirect dispersal estimate for males because the iterative process did not converge. This may be due to insufficiently powerful genetic markers for reliable relatedness estimates (Vekemans & Hardy 2004). The high level of heterozygosity in these markers (Kronauer *et al.* 2006), including in the Monteverde population (Soare *et al.* in press), suggests that the seven loci are powerful to detect patterns of relatedness among individuals. If males disperse farther than 1 km on average, then our spatial extent may be less than the ideal 10σ – 50σ, which would constrain our capacity to obtain an indirect dispersal estimate (Fenster *et al.* 2003; Vekemans & Hardy 2004). However, previous studies have detected spatial genetic structure in queens and males in lowland Panama within 10 km (Pérez-Espona *et al.* 2012; Soare *et al.* in press). Alternatively, any existing SGS in Monteverde may not be representative of an IBD process, and thus would constrain our ability to obtain indirect dispersal estimates (Vekemans & Hardy 2004).

The decline of queen pairwise relatedness with Euclidean distance was not significant, suggesting that queen dispersal is facilitated through colony nomadic behavior over time. The sample size of the current study (40 colonies) is the largest to date for examination of fine-scale SGS in *Eciton burchellii army* ants (Berghoff *et al.* 2008; Pérez-Espona *et al.* 2012; Soare *et al.* in press). Though army ant queen dispersal was found to be limited in lowland Panama (Berghoff *et al.* 2008; Pérez-Espona *et al.* 2012), these studies were carried out on an island and peninsulas created by the construction of the Panama Canal, and those landforms may restrict colony movements. The relatively recent history of development in the Monteverde region (Nadkarni & Wheelwright 2000) may mean that the population is not at drift-migration equilibrium. However, SGS was detected within one generation in simulations (Landguth *et al.* 2010; Banks & Peakall 2012).

The parentage analysis did not identify any direct parent-offspring relationships between cohorts, obstructing our efforts to compare male flight distance with queen dispersal through colony emigrations. Based on our estimate of local population density, we sampled about a quarter of the Monteverde population, and thus we should have detected a few direct dispersal events. Daughter colonies may leave the immediate area (disperse at least 500 m), limiting our ability to find offspring of the 2006 queens. A colony was sampled only 572 m away from the sampling location of 3 years previous. This distance is only slightly greater than the average distance covered over a single nomadic phase (529 m: Franks & Fletcher 1983; 489.5 m: Willson *et al.* 2011). Three years of nomadic phase movements correspond to expected travel of 3 km (random walk in a preferred direction: Franks & Fletcher 1983) or 16 km (straight line). Though colony behavior in the intervening three years is unknown, movements could have been constrained by deforestation and nearby development (Meisel 2006; Kumar & O'Donnell 2009; Pérez-Espona *et al.* 2012; Soare *et al.* in press), forcing the colony to remain in the forest fragment.

Direct measures of dispersal (i.e. re-sighting) differ from indirect measures (i.e. analysis of SGS) by a factor of two, and this is typically attributed to undetected long-distance dispersal events by offspring (Waser & Elliott 1991; Watts *et al.* 2007; Shanks 2009). Dispersal distances usually have a leptokurtic distribution (Waser & Elliott 1991; Watts *et al.* 2007), so it may be most probable for a short dispersal event to be the one captured in Monteverde army ants. The re-sampled colony was found 572 m from the initial collection location, and queens were significantly unrelated to queens in the same area three years previous. This suggests that queens disperse at least 500 m. Comparisons across cohorts revealed no spatial pattern of relatedness over time, suggesting that colony movements contribute to gene flow in army ant populations. Dispersal in army ants has been assumed to be male-biased (Berghoff *et al.* 2008; Jaffé *et al.* 2009; Pérez-Espona *et al.* 2012), though the difference in dispersal distance for queens and males may less than previously believed. Weak differences in sex-biased dispersal are difficult to detect (Goudet *et al.* 2002; Busch *et al.* 2009), as seems to be the case for army ants (Soare *et al.* in press; current study).

Though deforestation was found to inhibit (and mature forest promote) gene flow in army ants in previous studies (Pérez-Espona *et al.* 2012; Soare *et al.* in press) and in both sampling years combined in the current study, resistance distances based on land cover did not predict pairwise relatedness among queens in the 2009 cohort. The pattern of SGS was also not uniform across sampled cohorts. A lower sample size in 2009 may underpower efforts to detect the effects of landscape on army ant dispersal. We had more difficulty locating colonies in 2009. If a decline in samples represents a decrease in population density, this could inhibit attempts to detect SGS or obtain an indirect estimate of dispersal (Broquet *et al.* 2006). In crickets, cohorts with lower population densities displayed less fine-scale spatial genetic structure (Bretman *et al.* 2011). Fine-scale SGS also broke down in red deer (Nussey *et al.* 2005) and kangaroo rats (Busch *et al.* 2009) with changes in population density. Army ant colony density is lower in human dominated landscapes (Meisel 2004), and thus could have decreased with any increase in development in the Monteverde area between sampling years.

The observed difference in SGS across sampled cohorts underscores the need for replication in landscape genetics. Spatial replications have found that the effect of landscape features on gene flow is greatest in areas where those features are highly variable (Short Bull *et al.* 2011), and that estimates of SGS vary widely among sites (Jump *et al.* 2012; Soare *et al.* in press). Fine-scale SGS has also been found to be variable over sampling years, likely due to changes in environmental conditions (Nussey *et al.* 2005; Busch *et al.* 2009; Bretman *et al.* 2011; Messier *et al.* 2012).

Colony movements over the lifetime of the queen may facilitate gene flow in army ants and habitat connectivity should be maintained to promote such dispersal. Other animal behaviors such as habitat and diet specialization (Pilot *et al.* 2006; Pilot *et al.* 2012; Stevens *et al.* 2012), previous experience (Forero *et al.* 1999; Serrano *et al.* 2001), and social rank (Wittemyer *et al.* 2008) affect animal movements and subsequent gene flow. Though generalist predators (Gotwald 1995), *Eciton burchellii* army ants specialize on high density patches (Kaspari *et al.* 2011), which might select for longer dispersal distance (Johnson & Gaines 1990; Neville *et al.* 2006; Busch *et al.* 2009). Gene flow is vital for population persistence (Saccheri *et al.* 1998; Turelli *et al.* 2001; Broquet and Petit 2009). Thus it is important to maintain habitat connectivity to sustain genetically diverse populations of *Eciton burchellii* army ants and the myriad animal associates which the ants support (Rettenmeyer *et al.*2011).

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Tables

Table 1 Examination of sex-biased dispersal in army ants (pairwise kinship, F_{ij}) by year. Males displayed significant spatial genetic structure in 2009 and when both sampling years were combined. Sample size (*N*) is the number of pairwise comparisons (not including within-colony comparisons for males). Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values indicate significant relationships.

	Queens				**Males**			
year	**N**	***b***	**r^2**	***P***	**N**	***b***	**r^2**	***P***
2006	300	-0.0022	0.0004	0.687	28999	-0.0007	0.00001	0.684
2009	120	-0.0136	0.0195	0.224	**14129**	**-0.0069**	**0.0012**	**0.019**
combined	420	-0.0061	0.0031	0.218	**43128**	**-0.0030**	**0.0002**	**0.012**

Table 2 Effects of landscape features on army ant dispersal (pairwise relatedness, r_{ij}, among queens). Resistance distances based on land cover predicted patterns of relatedness in 2006 and when both sampling years were combined. Slope (*b*), coefficient of determination (r^2) and significance level (*P*, two-tailed) demonstrate strength of relationships. Bold values represent significant relationships (B-Y FDR adjusted alpha = 0.0161).

	2006			**2009**			**combined**		
model	***b***	***r^2***	***P***	***b***	***r^2***	***P***	***b***	***r^2***	***P***
2. Land cover	**-0.002**	**0.0469**	**0.005**	0.0024	0.0106	0.546	**-0.0017**	**0.0283**	**0.010**
3. Elevation	**0.0025**	**0.0247**	**0.015**	-0.0013	0.0060	0.552	0.0013	0.0066	0.148
4. Land cover + elevation	-0.0006	0.0052	0.323	-0.0006	0.0028	0.682	-0.0006	0.0060	0.191
5. Elevation band	0.0024	0.0067	0.202	-0.0033	0.0172	0.289	0.0005	0.0003	0.761
6. Land cover + elevation band	-0.0013	0.0232	0.039	-0.0003	0.0005	0.842	-0.0011	0.0161	0.036

Figures

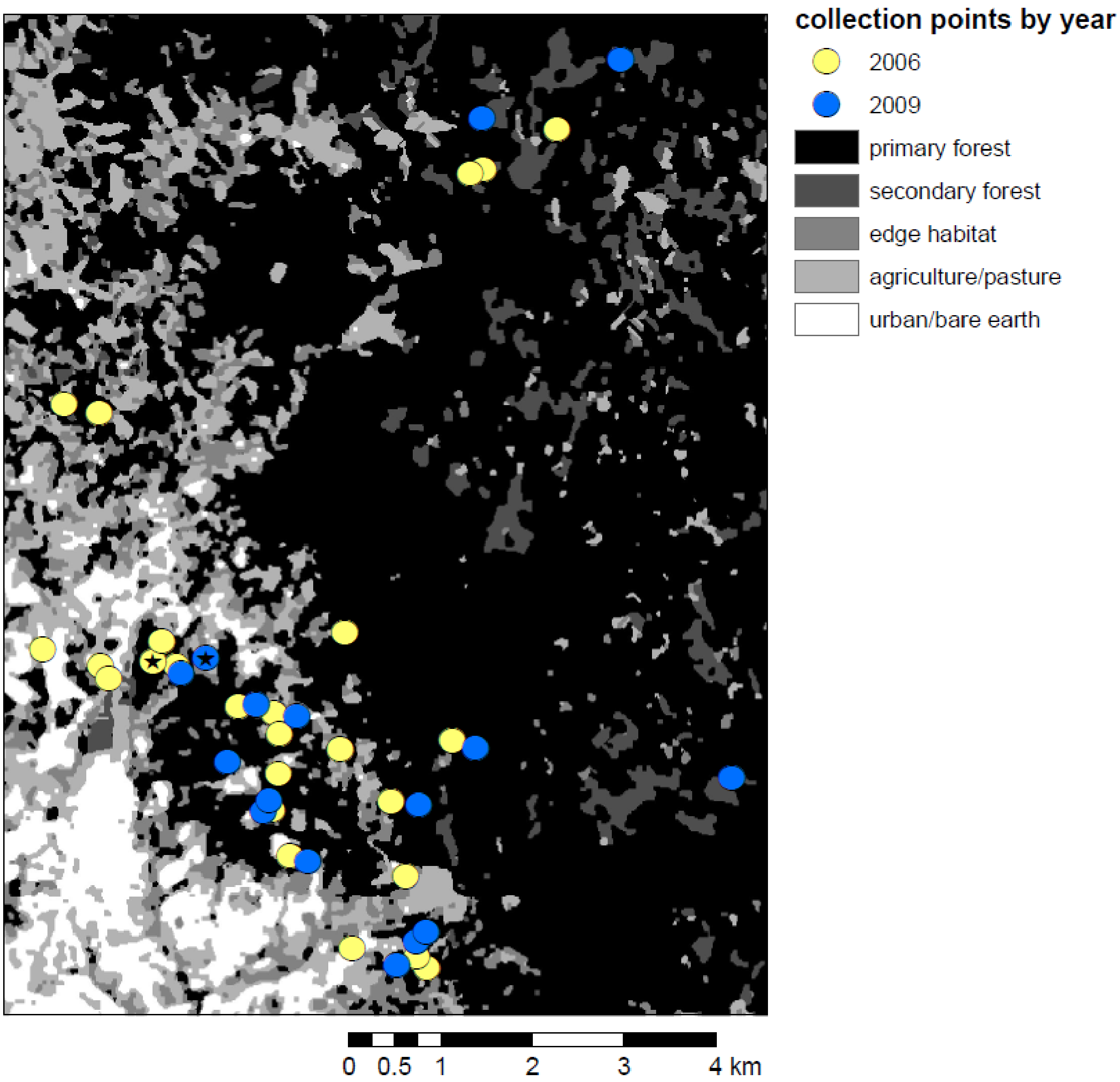

Fig. 1 Location of study site and map of land cover classes with locations of sampled colonies. Stars indicate locations of re-sampled colony in 2006 (yellow) and 2009 (blue). Five land cover classes (friction values) are primary forest (10), secondary forest (30), edge habitat (50), agriculture/pasture (70), urban/bare earth (100). Friction values are estimates of habitat resistance with 100 being a complete barrier to dispersal.

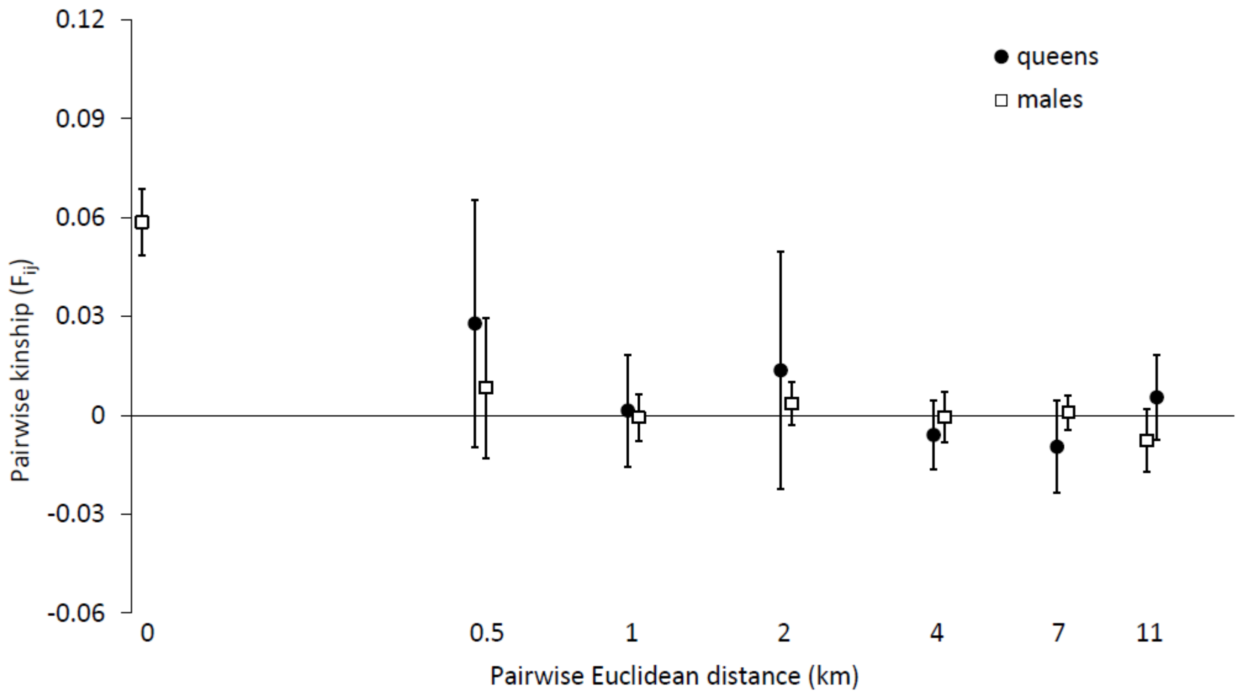

Fig. 2 Spatial genetic structure (SGS) within sampling years for each sex. Male, but not queen, pairwise kinship (Fij) significantly decreases as distance among pairs increases. A queen's mates are significantly related. Distance intervals are 0 (within colony), 0-0.5 km (not including within colony), 0.5-1 km, 1-2 km, 2-4 km, 4-7 km, and 7-11 km, graphed on the natural logarithm of Euclidean distance. Error bars represent +/- 2SE. Values offset to facilitate visualization.

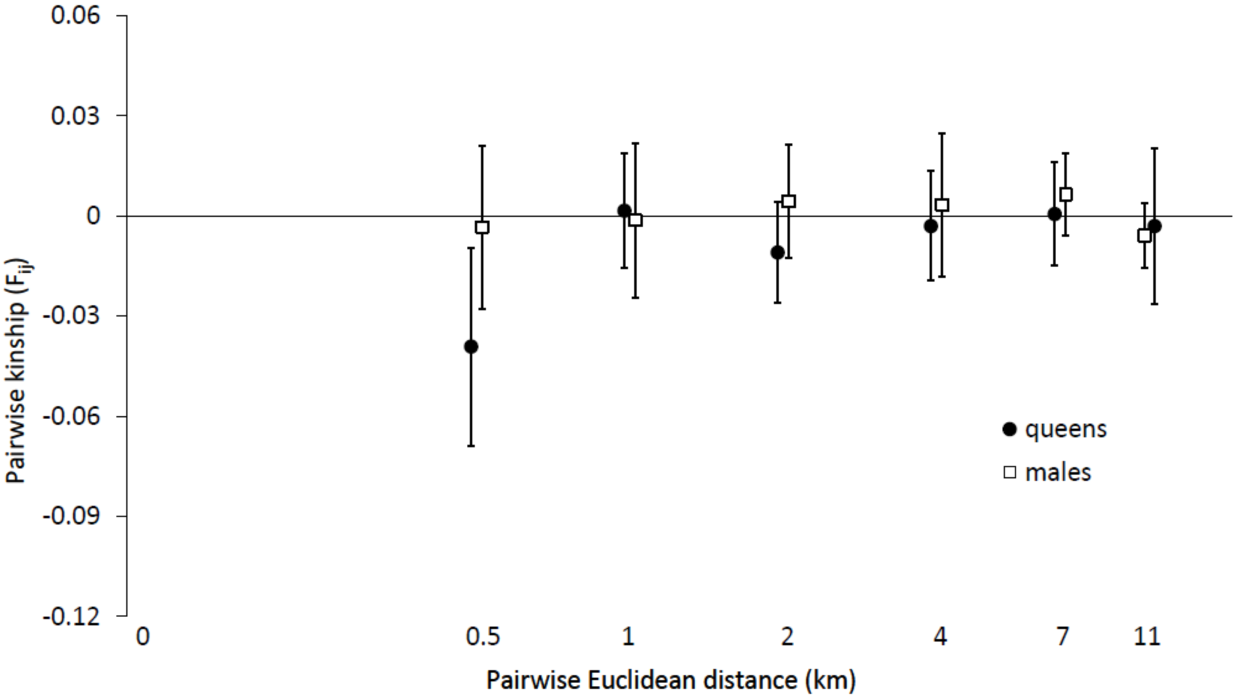

Fig. 3 Spatial genetic structure (SGS) across sampling years for each sex. Queens sampled in 2009 queens (re-sampled queen removed) are unrelated to queens sampled in 2006 in the same area (within 0.5 km). There is no pattern of relatedness of males sampled in 2009 to queens sampled in 2006. Distance intervals are 0-0.5 km, 0.5-1 km, 1-2 km, 2-4 km, 4-7 km, and 7-11 km, graphed on the natural logarithm of Euclidean distance. Error bars represent +/- 2SE. Values offset to facilitate visualization.

VITA

Thomas Warren Soare was born and raised in Jericho, NY. He earned a B.A. in Environmental Science, Ecological Track from Boston University in 2006. He earned a Ph.D. in Psychology (Animal Behavior) from the University of Washington in 2013. In addition to a very supportive family, he was fortunate enough to have many excellent teachers and friends encourage him along the way.