High indirect fitness benefits for helpers across the nesting cycle in the tropical paper wasp *Polistes canadensis*

Robin J. Southon[1#], Emily F. Bell[1#], Peter Graystock[1,2], Christopher D. R. Wyatt[1,3,4], Andrew N. Radford[1], Seirian Sumner[1,5*]

[1]*School of Biological Sciences, University of Bristol, Bristol BS8 1TQ, UK*

[2]*Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot SL5 7PY, UK*

[3]*EMBL/CRG Systems Biology Research Unit, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain*

[4]*Universitat Pompeu Fabra (UPF), 08002 Barcelona, Spain*

[5]*Centre for Biodiversity and Environmental Research, Department of Genetics, Evolution and Environment, University College London, London WC1E 6BT, UK*

Keywords: inclusive fitness, *Polistes*, relatedness, reproductive skew, single-nucleotide polymorphisms (SNPs)

[#]Equally contributing authors

*Corresponding author: s.sumner@ucl.ac.uk

Running header: Indirect fitness in a tropical paper wasp

Abstract

Explaining the evolution of helping behaviour in the eusocial insects where non-reproductive ('worker caste') individuals help raise the offspring of other individuals, remains one of the most perplexing phenomena in the natural world. *Polistes* paper wasps are popular study models, as workers retain the ability to reproduce: such totipotency is likely representative of the early stages of social evolution. *Polistes* is thought to have originated in the tropics, where seasonal constraints on reproductive options are weak and social groups are effectively perennial. Yet, most *Polistes* research has focused on non-tropical species, where seasonality causes family groups to disperse; cofoundresses forming new colonies the following spring are often unrelated, leading to the suggestion that direct fitness through nest inheritance is key in the evolution of helping behaviour. Here we present the first comprehensive genetic study of social structure across the perennial nesting cycle of a tropical *Polistes* – *Polistes canadensis.* Using both microsatellites and newly-developed single-nucleotide polymorphism (SNP) markers we show that adult cofoundresses are highly related, and that brood production is monopolised by a single female across the nesting cycle. Non-reproductive cofoundresses in tropical *Polistes* therefore have the potential to gain high indirect fitness benefits as helpers from the outset of group formation, and these benefits persist through the nesting cycle. Direct fitness may have been less important in the origin of *Polistes* sociality than previously suggested. These findings stress the importance of studying a range of species with diverse life-history and ecologies when considering the evolution of reproductive strategies.

1. INTRODUCTION

Societal living entails both benefits and costs for individuals (Krause & Ruxton, 2002). Unless clonal, one such cost is the conflict over reproduction that exists between group members when only some individuals produce young (West et al., 2002). In extreme cases, only one or a few group members reproduce whilst other (non-reproducing) individuals assist in rearing the young. Such reproductive division of labour, with high reproductive skew, is found in a range of social taxa (Wilson, 1971; Kokko & Johnstone, 1999; Cant & Johnstone, 2008). Determining how reproduction is divided amongst conspecific group members, and why individuals forgo independent breeding to be a helper, is fundamental for understanding the evolution of sociality (Keller & Reeve, 1999; West et al., 2007).

Non-reproductive group members may help because of future direct and/or current indirect fitness benefits. Where group relatedness is low, or less than the population average, helping can be explained by immediate or delayed direct fitness benefits. These include the advantages accrued from group augmentation and the potential to inherit the breeding position or territory (Clutton-Brock, 2009). When group members are closely related, the costs of helping can be balanced by indirect (kin-selected) fitness benefits (Queller & Strassmann, 1998), as proposed by Hamilton (1964). Changes in group composition that alter average group relatedness have potential implications for the relative payoffs of helping versus reproducing. Such changes can arise due to both intrinsic (e.g. intra-group competition and turnover of breeders) and extrinsic (e.g. seasonal constraints and fluctuating resource availability) factors (McCracken & Wilkinson, 2000; Koštál, 2006; Armitage, 2007; Wittemyer et al., 2007). Tests of how environmental conditions influence reproductive options, and the resulting fitness payoffs, are needed for a full understanding of the relative importance of direct and indirect fitness for helpers (Bourke, 2014).

The simple societies of *Polistes* paper wasps, where there is a division of labour in behavioural roles but all individuals (to a degree) retain the potential to reproduce, likely exhibit similar traits

to those found in the early stages of eusocial evolution (West-Eberhard, 1969; Jeanne, 1980; Reeve, 1991; Danforth & Eickwort, 1997; Keller, 2003). Studies to date have indicated the importance of both direct and indirect fitness benefits for *Polistes* sociality (Queller et al., 2000; Boomsma, 2007; Leadbeater et al., 2011; Field & Leadbeater, 2016). For example, in the best-studied species of the genus, *Polistes dominula*, direct fitness via nest inheritance has been shown to explain helping behaviour during the group-founding (foundress) stage, when group members are often unrelated (Strassmann et al., 1989; Kokko & Johnstone, 1999; Queller et al., 2000; Zanette & Field, 2008; Leadbeater et al., 2011; Field & Leadbeater, 2016). By contrast, indirect fitness from raising siblings explains helping behaviour in later stages of the nesting cycle of *P. dominula*, where the mothers of the helpers monopolise reproduction (Peters et al., 1995; Queller et al., 1997; Field et al., 1998; Queller & Strassmann, 1998; Reeve & Keller, 2001; Seppä et al., 2002). However, *P. dominula* is a temperate species where mated 'new queens' undergo an obligate winter diapause (or quiescence); this may have an impact on group dynamics, indirect fitness payoffs to subordinate cofoundresses in founding nests may be uncertain, because it is difficult for females emerging from diapause to find and select relatives with whom to found a nest (Queller et al., 2000; Starks, 2003; Dapporto et al., 2004; Zanette & Field, 2008; Seppä et al., 2012; Field & Leadbeater, 2016). In the tropics, insects are not subject to the same overwintering diapause as their temperate counterparts (Denlinger, 1986). This means that social groups in tropical Hymenoptera can be essentially perennial as their nesting cycles are asynchronous, and reproductively potent individuals can initiate new nests and mate at any time of the year (West-Eberhard, 1969; Pickering, 1980; O'Donnell & Joyce, 2001). Therefore, females in tropical species potentially have access to better information (and different mechanisms) for selecting relatives to nest with than do temperate species (Dani et al., 2004; Dapporto et al., 2004; Sumana et al., 2005; Tibbetts & Injaian, 2013). *Polistes* is thought to have originated in the tropics (Carpenter, 1996; Santos et al., 2015), and two-thirds of species in this genus are tropical (West-Eberhard, 1969). The impact of climate and environmental variation on sociality has recently been noted in cooperatively

breeding birds, bees, and wasps (Richards, 2000; Fucini et al., 2009; Field et al., 2010; Cockburn & Russell, 2011; Cronin et al., 2011; Jetz & Rubenstein, 2011; Sheehan et al., 2015). However, we lack detailed analyses of the genetic structure and fitness strategies employed in *Polistes* species across latitudinal gradients, especially for the foundress period and tropical species (see summary in Appendix S1). Specifically, genetic data on foundress nests are limited to seven species (four species in temperate regions, three in subtropical), and entirely miss tropical species. This is a critical gap in our understanding of social evolution as it is the tropical – as opposed to temperate – *Polistes* that are likely to experience ecological conditions similar to those under which sociality first evolved in this genus.

Here we examine genetic structure and reproductive partitioning in societies of the tropical paper wasp *Polistes canadensis*, an emerging model species for *Polistes* research (Jeanne, 1979; Pickering, 1980; West-Eberhard, 1986; Sumner et al., 2006; Sumner et al., 2007; Sumner et al 2010; Ferreira et al., 2013; Patalano et al., 2015). New nests are cofounded by several females (Pickering, 1980; Miller et al., 2018), yet it is unknown whether these females are often of low relatedness or unrelated, as in temperate species (Appendix S1). The República de Panamá, in the central range for *P. canadensis*, experiences seasonal variation in the form of wet and dry periods, but there is no enforced overwintering diapause period. All stages of the nesting cycle can be found together throughout the year (including the dry season) (Pickering, 1980), permitting their simultaneous study under the same environmental conditions. These life-history and environmental traits allow us to investigate whether species that have weak seasonally-induced changes in group membership consist of highly related cofoundresses. Closely related cofoundresses are predicted to exhibit little conflict over reproduction as the indirect fitness payoffs from helping will be high. Support for this prediction would have important implications for our understanding on the evolution of helping in environments that are most similar to those of in which the social behaviour of *Polistes* first evolved. Specifically, it would imply that direct fitness (as seen via nest inheritance in

temperate species) may be less important in the early stages of social evolution than previously thought.

To test this prediction, we identified a set of single-nucleotide polymorphism (SNP) markers from transcriptome data; these markers permit the fine-scale analyses of genetic structure data required to assess fitness payoffs, but also present an opportunity to compare results obtained via more traditional markers (e.g. microsatellites). We used these SNP markers and previously published microsatellite markers (Lengronne et al., 2012) to genotype adults and brood from 53 nests from across the nesting cycle in order to test three predictions. Prediction 1: cofoundresses nest in mother-daughter or sister-sister groups, thus maximising potential indirect fitness payoffs from helping – suggesting that mechanisms exist by which females are able to select relatives to found new nests with or that philopatry generates favourable conditions for helping. Prediction 2: there is an effective monopoly of reproduction (i.e. high reproductive skew) in foundress nests – implying that there is the potential for high indirect fitness payoffs for cofounding helpers, if related. Prediction 3: a reproductive monopoly is perpetuated in established post-emergence nests – this would assure long-term indirect fitness payoffs for helpers (Figure 1).

2. METHODS

2.1. SNP-marker discovery and validation

SNPs were identified from transcriptome RNA-seq assemblies available for 10 individuals of *P. canadensis* (Patalano et al., 2015), and for unpublished transcriptome sequences for 10 individuals of a sister species *Polistes lanio* – the latter collected at Verdant Vale, Blanchisseuse Rd, Trinidad, Trinidad & Tobago (10°41'5.44"N, 61°17'24.95"W) (Sumner et al. unpublished). Raw RNA-seq reads were mapped against the *P. canadensis* genome (Patalano et al., 2015) using the Burrows–Wheeler Aligner to obtain binary alignments maps (BAMs), then sorted using SAMtools (Li et al., 2009). SNPs were called using 'mpileup' and 'view' functions within the BCFtools package (Li, 2011).

To generate a flexible SNP resource for *Polistes*, we chose SNP sites that were biallelic in both *P. canadensis* and *P. lanio*. Quality filtering of candidate SNPs was performed by visualising against RNA-seq assemblies of both species using Integrative Genomics Viewer (IGV) (Robinson et al., 2011; Thorvaldsdóttir et al., 2013). SNPs with low allele frequencies (<20%) or a sequencing depth fewer than 150 reads were filtered out. As an additional quality filter, and to aid the reliability of the downstream KASP™ (LGC Genomics) genotyping assays, only SNPs located more than 80 bases from one another or to the end of a scaffold were selected (proximity filtering). From this process, 120 biallelic SNPs were selected across a range of scaffolds with the highest coverage.

A conservative filtering method was used to select a set of 69 polymorphic SNP loci for KASP™ SNP genotyping from the 120 candidate SNPs. To identify a set of loci that were reliably polymorphic for this species and study population, initial validation using 120 identified loci was performed on a subset of 55 individuals from across five post-emergence nests (see Section 2.4). Loci were selected through manual examination of clusters, removing loci with monomorphic or indeterminate allelic clustering, along with removal of any loci with Minor Allele Frequencies (MAF) <5%. Individual ambiguous allele calls were removed to reduce genotyping errors (Semagn et al., 2014). Subsequently, 69 loci were selected for the full genotyping

project. Linkage disequilibrium (log likelihood-ratio statistic; 1000 dememorizations, 100 batches, 1000 iterations per batch), deviations from Hardy-Weinberg equilibrium (Bonferroni corrected $\alpha = 0.0007$; 10,000 Monte Carlo permutations), and heterozygosity (Bartlett's test) were assessed using 41 females from separate nests (see Section 2.2) – using the R packages 'adegenet' (Jombart, 2008; Jombart & Ahmed, 2011), 'genepop' (Rousset, 2008), and 'pegas' (Paradis, 2010).

2.2. Genetic structure sample collection

Foundress and post-emergence nests of *P. canadensis* from a range of comb (the paper nest) sizes and brood compositions were analysed. Foundress nests were defined as those containing only eggs or small larvae, with no evidence of emerged adults (i.e. hatched pupae caps). Post-emergence nests were defined as those with a mixture of developing brood (eggs, larvae, and pupae), and evidence of hatched pupae caps.

All nests were collected in May–August 2013 from four abandoned buildings at a 4 hectare site at Fuerte Sherman, Colón Province, República de Panamá (9°21'42.57"N, 79°56'58.49"W). Twenty-nine nests were selected for SNP (11 foundress; 10 post-emergence nests) or microsatellite (8 foundress nests) genotyping; all nests consisted of a single comb. Prior to the collection of post-emergence nests, an egg-layer and a forager were identified from behavioural observations and collected, to verify predicted parentage, without depleting the population. The egg-layer was identified on each nest by removal of an egg in a cell and observing which individual subsequently oviposited within an hour. A worker was identified through *ad libitum* observation, noting if a wasp brought a solid mass of forage to the nest; sampling a worker enabled us to assess the longevity of a queen/matriline, without having to destroy 'nests' completely (remaining nestmates can rebuild their nests). To enhance statistical power in SNP-marker validation (see Section 2.1), a single female was collected from each of

20 additional post-emergence nests on the site that were not used in the study in any other way. All samples (adults and brood) were collected at dusk, when most foraging wasps are likely to have returned to the nest. Samples were stored in 80% EtOH at -20°C. The number of cells and brood (categorised as eggs, larvae, or pupae) were counted in all sampled nests (Appendix S2). A further 24 post-emergence nests (genotyped at the same microsatellite loci) were reanalysed from Lengronne et al. (2012) to augment the sample size for the pedigree analysis (see Section 2.6). These nests were collected from a second population, about 9.6 km away from Fuerte Sherman (see Lengronne et al., 2012).

2.3. Assessment of reproductive state

All genotyped adult females were dissected under a microscope to estimate reproductive status (Cini et al., 2013). Ovarian development of each individual was assigned to one of five categories (adapted from Gobbi et al., 2006): A = small filamentous ovarioles lacking oocytes; B = small ovarioles with slightly developed oocytes; C = large ovarioles with few developed oocytes at the base of tract; D = large ovaries with multiple fully developed oocytes; E = large ovaries with signs of regression (e.g. yellow-bodies). The length of the largest oocyte in the reproductive tract was recorded in millimetres, and considered a mature egg if longer than 2 mm (Sumner et al., 2006). Reproductively capable females were defined as having mature eggs (category C–E), and actual egg-laying females as having mature eggs and a maternal genotype that is compatible with being the mother of the brood's genotypes (see Section 2.6) (Cini et al., 2013). A Wilcoxon signed-rank test (two-tailed), comparing the number of individuals per nest with mature eggs and those without, was used to analyse whether subordinate cofoundresses (i.e. those without genotyped offspring on the nest) were more likely to be primed for reproduction than not.

2.4. SNP genotyping

Genetic structure in foundress and post-emergence nests was analysed using the new SNP markers (69 SNP loci). For 10 foundress nests, all female adults and 25–80% of eggs (sampled from across the comb) were genotyped (65 adults and 72 eggs in total). In an additional 11th foundress nest, all four female adults were genotyped, but brood was not. For the 10 post-emergence nests, the reproductive and foraging females that were identified from observations (see Section 2.2), together with five eggs and up to five pupae of each sex per nest (10 reproductives, 10 foragers, 50 eggs, 50 female and 39 male pupae total) were selected. Assessing maternity of eggs and pupae within the same nest can reveal whether multiple females are laying eggs, and whether (for example) the brood of secondary egg-layers are subsequently removed via selective oophagy such that the realised direct fitness of subordinate egg-layers is zero. For the post-emergence nests, eggs and pupae were sampled from across the nest by dividing a comb into quarter grids, and selecting across grids in sequence until the required sample size was collected. Sampling across the comb in this way accounts for different egg-layers in specific areas of the nest (as suggested in West-Eberhard, 1986). Adults and pupae were sexed by counting the number of antennal flagellum segments; those with 10 segments scored as females and those with 11 as males (Strassmann et al., 2003).

Prior to DNA extraction, samples were washed in 90% EtOH to reduce the chance of foreign DNA contamination on the exoskeleton (Shokralla et al., 2010), and allowed to dry at room temperature. For adults and pupae, 2 mm of coxa was dissected and broken apart to reveal tissue. Eggs were removed intact from the comb. DNA was extracted using a HotSHOT protocol (Truett et al., 2000; Montero-Pau et al., 2008), with individual coxae and eggs added to 50 µl alkaline lysis buffer (NaOH 50 mM, disodium EDTA 0.4 mM, pH 12.0) and heated to 95°C for 2 h. To neutralise, 50 µl of Tris-HCl 40 mM pH 5.0 was added, and a solution created of 35 µl DNA extract diluted with 100 µl H_2O. Extracted DNA was stored at 4°C (short-term) or -20°C (long-term) before use in PCR reactions.

SNP genotyping was conducted using KASP™ genotyping assays. Reaction master mixes consisted of 11.83 µl custom KASP™ assay mix, 422.4 µl KASP™ V4.0 2x Master Mix v4.0 low ROX™, and 422.4 µl H_2O added in 8 µl aliquots to 2 µl of each extracted DNA sample, and spun for 4 min at 2500 rpm. Thermal cycling was performed with an Agilent Mx3005P qPCR System and consisted of 94°C for 15 min (1 cycle); 94°C for 20 s and 61°C for 1 min (10 cycles); 94°C for 20 s and 55°C for 1 min (35 cycles); finally, 30°C for 1 min. Pre- and post-cycle fluorescence was read at 25°C, with dyes HEX™, FAM™, and ROX™ reference dye, normalised and plotted in MxPro™ Mx3005P® v4.10. Between 0 and 5 additional cycles of 94°C for 20 s and 55°C for 1 min were performed after manually evaluating 35 cycle touchdown post-PCR reads. Each PCR plate included at least three negative (no DNA) and two positive (duplicates for initial runs, repeats for subsequent runs) controls. Pre-extraction sexing of adults and pupae allowed checking of genotyping accuracy through sex-specific hemi- (male) and hetero- (female) zygosity of the haplodiploid genetic system that Hymenoptera exhibit. No diploid males were detected amongst pupae (Liebert et al., 2004); the criteria set of <2% heterozygosity across loci in a sample was used to identify male eggs (all detected males had zero heterozygosity).

2.5. Microsatellite genotyping

We used a set of seven proven polymorphic microsatellite markers (Multiplex 1: Pcan01 HEX™, Pcan15 6-FAM, Pcan23 6-FAM; Multiplex 2: Pcan05 HEX™, Pcan09 HEX™, Pcan16 6-FAM, Pcan24 6-FAM – Lengronne et al., 2012) to genotype all female adults from eight foundress nests (36 adults total). In addition, we analysed the pedigree structure of 24 post-emergence nests from Lengronne et al. (2012) (same loci; five female pupae per nest, 120 pupae total). Lengronne et al. (2012) previously reported on the mean relatedness of these post-emergence nests, and here we reanalyse to discover parentage of the brood.

Foundress adult DNA was extracted by evaporating storage ethanol before mashing individual thoraxes in 10 µl Proteinase K and 250 µl DIGSOL, then incubating in a 55°C water bath overnight. Then 300 µl 4 M ammonium acetate was added, vortexed (13000 rpm), washed in 99% and 70% EtOH, and the supernatant retained. Samples were finally air-dried, 50 µl of low TE buffer added, and placed in a 37°C water bath for 30 minutes. Extracted DNA was stored at 4°C (short-term) before use in PCR reactions.

PCR protocol consisted of 8 µl reaction mixes of 2.4 µl of multiplex (0.3M), 4 µl Qiagen *Taq* PCR Master Mix, 0.4 µl H_2O, and 1.2 µl of extracted DNA sample in a 96-well plate; centrifuged before 10 µl mineral water added to the top of each well. Thermal cycling of 95°C for 15 min (1 cycle); 94°C for 30 s, 60°C for 1 min 30 s, and 72°C for 1 min (35 cycles); finally, 60°C for 30 min (1 cycle). Each PCR plate included at least three negative (no DNA) and two positive (duplicate) controls. Sequencing of 5 µl diluted PCR product was performed at University College London, using ROX™ reference dye. Microsatellite scoring was conducted in Geneious® 11.1.4.

2.6. Nesting-group genetic structure analyses

Female relatedness was estimated using COANCESTRY 1.0.1.8 (Wang, 2011), reporting the Wang (2002) relatedness estimation. Individual inbreeding coefficients *F* were derived from the Lynch & Ritland (1999) estimate calculated in COANCESTRY, and tested for significant differences from a coefficient of zero (random mating) using one-sample t-tests.

Adults and brood were assigned to direct matrilines (mother and sib-groups), and the genotypes of their putative mothers (matrilines) and fathers (patrilines) were predicted based on allele sharing, using COLONY 2.0.6.5 (Jones & Wang, 2010; settings: female polygamy,

male monogamy, without inbreeding, haplodiploid, very long run length, full-likelihood, no updated allele frequency, and sibship prior none). We ran multiple models to determine pedigree: (1) a preliminary model of all foundress adults to all foundress brood (medium run length, Appendix S9); (2) a model of potential foundress mothers to all foundress 'offspring' (i.e. individuals identified in the preliminary model as mothers, with offspring being those adults and brood excluded as mothers) (Appendix S10); (3) a model of all foundress adults as 'offspring' (to confirm or reject mothers of 'brood-only' as sisters of adult nestmates) (Appendix S10); (4) a model of confirmed post-emergence mothers (through egg removal) to the single adult worker and brood (Appendix S10); (5) a model of microsatellite post-emergence pupae as offspring (Appendix S10). Assignment of clusters was accepted only when either the probability of assignment was ≥0.80 or substructure analyses showed large differences between secondary structures. The possibility of polyandry in matriline assignment was allowed, as our preliminary analyses (see Appendix S11) and previous microsatellite analyses of spermatheca (Sumner et al. unpublished) suggests low levels of multiple mating is present in *P. canadensis*.

Reproductive skew indices give a measure of reproductive partitioning among potential parents. The *B* index, a binomial index combining observed and expected variance (Nonacs, 2000), was used to measure reproductive skew of matrilines (i.e. in genotyped offspring). The *B* index has flexible assumptions and allows for small sample sizes (Nonacs, 2003). Calculated reproductive skew in a group may range from -1 (equally shared) to 1 (monopolised). The *B* index was determined in SKEW CALCULATOR 2003 (© Peter Nonacs) with 1000 simulations, 95% confidence intervals (CI), $\alpha = 0.05$ (see Appendix S12). The default setting of equal length of time spent in the nesting group per potential mother was assumed.

2.7. Mating frequency

The effective mating frequency of females k_{e3} (Nielsen et al., 2003) across all matrilines (i.e. number of fathers expressed in genotypes, whether singly or multiply mated), excluding any matrilines consisting of a single genotyped offspring, was calculated (reporting harmonic means and 95% CI).

All statistics, unless otherwise stated, were performed in R 3.3.3 (R Core Team, 2013) to $\alpha = 0.05$, reporting the mean (± standard error) or median (interquartile range, IQR).

3. RESULTS

3.1. SNP-loci discovery and validation

Using data from *P. canadensis* and *P. lanio* we detected 20,402 SNPs, of which 1,790 were heterozygous (Appendix S3). Amongst these SNPs, 918 had allele frequencies >20%, with an

even coverage against both *P. canadensis* and *P. lanio* assemblies. After proximity filtering, 202 high-quality SNP candidates remained. The final 120 SNPs were selected from these for validation, based on coverage (minimum 765 reads) and location across a range of 80 scaffolds (Appendix S4). Initial selection with KASP^{TM} genotyping (120 loci) identified 25 loci for removal that failed manual clustering evaluation; a further two loci were excluded due to MAFs <5%; another 24 loci were excluded due to poor genotyping clarity. This filtering left 69 loci. No significant deviations from Hardy-Weinberg were detected in these loci; all had MAFs ≥10%; linkage disequilibrium was observed across 107 loci pairings (4.6% of 2346 pairings). Genotyped samples had no discrepancy with positive controls. The percentage of successful manual allele assignment within samples was 80.1 ± 0.6% (in validated loci). These 69 loci were used for full genetic structure analyses in the 11 foundress and 10 post-emergence nests.

3.2. Nesting-group demographic and genetic characteristics

SNP and microsatellite analysed foundress nests consisted of 5.5 ± 0.6 adults (all female), with combs of 22.2 ± 2.6 cells. Post-emergence nests consisted of 23.1 ± 2.5 adults (mixed sex, at least a small proportion of adult males per nest), with combs of 231.4 ± 34.0 cells (Appendix S2). No male brood were detected in foundress nests. In eight out of the 10 SNP post-emergence nests male pupae were present, and male eggs were discovered (through genotyping) in all 10 post-emergence nests – representing 56.0 ± 8.8% of genotyped eggs.

Observed heterozygosity (H_{obs}) in 41 females (one female per nest) from the SNP dataset was 0.39 ± 0.02 (range: 0.00–0.61) across all loci, and not significantly different from expected ($H_{expected}$ 0.38 ± 0.01, range 0.00–0.50) (Bartlett's K-squared = 0.42, p = 0.516). The mean population (234 females) inbreeding coefficient F = -0.06 ± 0.01 (range: -0.43–0.36; lower/upper 95% CI range: -0.59–0.06/-0.28–0.63) and was significantly lower than 0 (t_{233} = -5.25, p < 0.001), suggesting some outbreeding. In eight adult foundress females (one female

per nest) in the microsatellite dataset H_{obs} was 0.68 ± 0.09 (range: 0.25–0.88), and significantly different from expected ($H_{expected}$ 0.65 ± 0.03, range 0.50–0.73) (Bartlett's K-squared = 5.78, p = 0.016). The mean population (36 females) inbreeding coefficient F = -0.04 ± 0.03 (range: -0.26–0.58; lower/upper 95% CI range: -0.66– -0.14/-0.14–1.00) and was not significantly different from 0 (t_{35} = -1.29, p = 0.207).

3.3. Testing the predictions

Prediction 1: cofoundresses nest in mother-daughter or sister-sister groups

Cofoundresses were close relatives. Mean relatedness (r) between adult female cofoundresses across all 19 foundress nests was 0.65 ± 0.04 (range: 0.26–0.81; Appendix S13). Relatedness of pairs of cofoundresses peaked at r = 0.8 (pairwise relatedness rounded to 1 d.p.; Figure 2; pairwise relatedness data given in Appendix S7), with a minor peak around r = 0.3, indicating that foundresses were only very occasionally less related than full sisters or mother-daughters. This pattern contrasts with the more prominent secondary peaks around r = 0.0–0.3 in frequency distributions of pairwise relatedness among *P. dominula* foundresses (Queller et al., 2000; Zanette & Field, 2008; Leadbeater et al., 2010). The type of marker (SNP or microsatellite) was relatively consistent: mean r between adult female cofoundresses was 0.70 ± 0.04 (SNPs, 11 nests) and 0.59 ± 0.08 (microsatellites, 8 nests).

COLONY analyses, which grouped individuals into sib-groups, revealed three types of family structure among adults in foundress nests (Appendix S13). Probability of assignment in both adults and brood was 1.00 ± 0.91. 'Sister' social structures best described the genetic structure in seven nests. Four nests (foundress nests #4–6 and #11) consisted of a single matriline of sisters (cofoundress adults, r = 0.81 ± 0.00). Three other nests (foundress nests #7–9) consisted of two matrilines (cofoundress adults, r = 0.54 ± 0.02; relatedness between different matriline adult offspring in these nests was r = 0.17 ± 0.08, 0.24 ± 0.02, and 0.33 ± 0.03 in

foundress nests #7–9 respectively). The second type of genetic structure was ‘Matrilineal’, in which a mother had apparently renested (e.g. left an established post-emergence nest on which she was likely to have been queen) with daughters. Matrilineal social structures were detected in three nests (foundress nests #1, #3, and #10; cofoundress adults, $r = 0.66 \pm 0.09$; daughters were related to their respective renesting mothers by $r = 0.57 \pm 0.12$). A single nest consisted of a mother and her three daughters, one of which was the primary egg-layer (foundress nest #2; cofoundress adults, $r = 0.77 \pm 0.04$; designated social structure ‘Mixed’) (Figure 3A). These nesting structures support our prediction that cofoundresses nest in mother-daughter or sister-sister groups.

Prediction 2: reproductive monopoly in foundress nests

Mean relatedness among brood (all female) within foundress nests was 0.72 ± 0.02 (range: 0.59–0.83) (Appendix S13). COLONY analysis confirmed a reproductive monopoly in foundress nests, with a single matriline detected in eight of the 10 nests analysed. A secondary egg-layer was detected in foundress nests #2 and #7, and in each case this extra egg-layer contributed a single egg (Figure 3B); in these nests, relatedness among brood was 0.59 and 0.75 respectively. In foundress nest #2, the secondary egg-layer was the mother of the other cofoundresses, with the primary egg-layer being a daughter (Mixed structure). In foundress nest #7, the secondary egg-layer was identified as one of the cofoundresses collected on foundress nest #8, suggesting a single female was laying on multiple nests. Reproductive monopoly was further confirmed from the high female reproductive skew index within foundress nests (B index $= 0.651 \pm 0.038$, $\alpha = 0.05$, range: 0.438–0.802).

Despite near reproductive monopoly among brood in foundress nests, a mean of $46 \pm 6\%$ of adult females per nest (19 nests) had mature egg/s present in their ovary tract (Appendix S8). In foundress nests with brood pedigree data: eight out of 10 females who matched the genotypes of the predicted egg-layers (10 nests) had ovaries with mature eggs, seven females

scored D and one C grade ovaries; the remaining two (no mature egg) females scored A and D. Adult cofoundresses who were not assigned as mothers (i.e. non-reproductive cofoundresses) exhibited a range of ovary grades from A to D (number with mature eggs = 20 females, 36%; A = 26 females, 47%; B = 14 females, 25%; C = 13 females, 24%; D = 2 females, 4%) (Figure 4A). No regressed (grade E) ovaries were discovered in any cofounders. Foundress nests (10 nests) did not differ significantly in the number of non-reproductive females (per nest) that had mature eggs (1.5, IQR = 2.8) or not (3.0, IQR = 2.5) (Wilcoxon signed-rank test: $W = 10.50$, $p = 0.169$) (Figure 4B).

No multiple mating was detected amongst genotyped eggs from ten foundress nests; however when cofoundresses were included as 'offspring', multiple mating was detected in three matrilines, with two males contributing to two of the matrilines and three males contributing to a third matriline; harmonic mean $k_{e3} = 1.09$ (lower/upper 95% CI: 0.93/1.25).

Prediction 3: reproductive monopoly is maintained in post-emergence nests

A single egg-layer was detected in all but one of 33 post-emergence nests, suggesting that high skew is maintained in these nests. Relatedness among female brood in 34 post-emergence nests was 0.69 ± 0.02 (range: 0.35–0.84; Appendix S14). In the SNP dataset (eggs and pupae, 10 nests), female brood to brood $r = 0.71 \pm 0.01$ (range: 0.64–0.79). In the microsatellite dataset (pupae, 24 nests), female brood to brood $r = 0.69 \pm 0.02$ (range: 0.35–0.84). COLONY analysis confirmed a reproductive monopoly in these nests (Appendix S10). Probability of assignment for male and female brood was 0.96 ± 0.04 (SNPs) and female pupae 0.96 ± 0.04 (microsatellites) (post-emergence nest Lengronne #23 excluded from pedigree, assignment probability being 0.23). The single genotyped adult forager was identified as the daughter of the dominant reproductive ($r = 0.51 \pm 0.02$) in each of the 10 post-emergence SNP nests, and these individuals were included as 'offspring' in COLONY analysis. Female brood were assigned to a single matriline in all but one nest, with this nest having a single female

pupa assigned to a second matriline (post-emergence nest #7). In the SNPs dataset, male brood were attributed to a single matriline in all 10 nests. In each case, the predicted genotype of the mother of the male eggs matched that of the mother of the sole matriline among the female brood. The predicted mother's genotype matched that of the queen identified from behavioural observations in all 10 SNP nests. This suggests that there is almost always a single egg-layer who monopolises production of both male and female brood in post-emergence nests. High female-reproductive skew indices across male and female brood confirmed the overall reproductive monopoly in all post-emergence nests (Overall *B* index = 0.785 ± 0.013, α = 0.05, range: 0.667–0.922; SNP dataset *B* index = 0.875 ± 0.021, α = 0.05, range: 0.682–0.922; microsatellite dataset *B* index = 0.745 ± 0.007, α = 0.05, range: 0.667–0.779).

The queens identified from behavioural observations in the post-emergence SNP nests all had D grade ovaries containing mature eggs (10 genotyped mothers). By contrast, only one forager (out of 10) had a mature egg in her ovary tract, with the most common ovary grade in foragers being A (A = 8 females, 80%; B = 1 female, 10%; C = 1 female, 10%; D = 0%). Regressed (grade E ovaries) were not found in either reproductive dominants or foragers.

Multiple mating was detected in matrilines of two post-emergence nests, harmonic mean k_{e3} = 1.04 (lower/upper 95% CI: 0.89/1.18). One mother had twice mated, another had mated three times; 33 matrilines total with more than one offspring; SNP dataset k_{e3} = 1.00; microsatellite dataset k_{e3} = 1.05 (lower/upper 95% CI: 0.88/1.23). Overall, across all the matrilines discovered in SNP and microsatellite genotypes in this study (53 nests; 48 matrilines – foundress and post-emergence), overall harmonic mean k_{e3} = 1.05 (lower/upper 95% CI: 0.96/1.15) (Appendix S15).

4. DISCUSSION

Here we have presented the first comprehensive analysis of the genetic-structure of a tropical *Polistes* wasp at two stages of the nesting cycle, and the first (to our knowledge) development and use of SNP-relatedness genotyping in a hymenopteran other than two economically important species and model organisms, namely *Apis mellifera* (honeybee) and the parasitic wasp *Nasonia vitripennis* (Whitfield et al., 2006; Niehuis et al., 2010). This represents a new molecular resource for genetic studies on *Polistes* wasps, as well as providing novel insights into the early stages of social evolution. Our analyses suggest that relatedness among foundresses is high (Prediction 1): females generally found new nests as a mix of matrilineal and/or sister groups, and groups of unrelated females were not widely detected. We also show that a reproductive monopoly is common at the foundress stage (Prediction 2), and that this is maintained in post-emergence nests in the reproduction of both female and male brood (Prediction 3). Our findings were supported in both SNP and microsatellite datasets, suggesting that these two methods are equally reliable and comparable. The potential payoffs from indirect fitness for totipotent non-reproductive females are therefore high throughout the nesting cycle. Our findings appear to contrast with similar studies on temperate *Polistes* species (Queller et al., 2000; Reeve et al., 2000; Leadbeater et al., 2011; Field & Leadbeater, 2016; see Appendix S1), in which cofoundresses are often of low relatedness (or unrelated) and thus direct reproduction (via nest inheritance) is thought to be an important component of fitness for helpers. Taken together, our study posits that indirect fitness is important for helpers in this tropical wasp from the outset of nest foundation. The tropical ecology of *P. canadensis* is likely to represent that in which group living first evolved in this lineage (Carpenter, 1996; Santos et al., 2015). We encourage similar analyses on other tropical *Polistes* (especially those in the 'Old World' tropics) to determine whether this is a general trait of tropical *Polistes.* These findings suggest the importance of considering how contrasting life-history and ecologies of

otherwise closely related species influence reproductive strategies, and that this should be considered when interpreting the origins of social evolution.

High relatedness between cofoundresses in *P. canadensis* offers high indirect fitness incentives to cofoundress helpers who do not reproduce. Non-reproductive cofoundresses are likely to be either raising nieces in Sister social groups (r = 0.375) or full sisters in Matrilineal social groups (r = 0.75). In contrast, in the well-studied temperate *P. dominula,* multiple studies report between 15–35% of cofoundresses being unrelated (Queller et al., 2000; Zanette & Field, 2008; Monnin et al., 2009; Leadbeater et al., 2011). The frequency distribution of the pairwise relatedness of cofoundresses is perhaps the most important result to compare here: in *P. canadensis* (this study, Figure 2) most pairs peak around r = 0.8, with a secondary peak around r = 0.3. In contrast, the frequency distribution of pairwise cofoundress relatedness in *P. dominula* peaks at zero as well as 0.75 (see Queller et al., 2000; Zanette & Field, 2008; Leadbeater et al., 2010). Based on these two species, it could be that temperate (e.g. *P. dominula*) foundress nests consist of full sisters but also pairs of unrelated or low-related (cousins) females, whilst tropical (e.g. *P. canadensis*) foundress nests consist mostly of mother-daughter and sister-sister pairs, with occasional pairs who are related but not full sisters or daughters of the egg-layer. Unfortunately, pairwise relatedness analyses of foundresses are primarily and currently limited to these two species and thus this pattern requires further study of species across climatic gradients. However, data on average cofoundress relatedness exist for three other temperate species (Appendix S1): in *Polistes fuscatus* mean relatedness of cofoundress was estimated at 0.57 ± 0.06, with 25% of foundress nests consisting of cousins or unrelated pairs (Reeve et al., 2000); average relatedness of cofoundresses in *Polistes aurifer* is very low (0.13 ± 0.08, Liebert et al., 2005); estimates for *Polistes metricus* cofoundresses (r = 0.63) are only available from allozyme data and are less precise (at five loci, Metcalf & Whitt, 1977). The high chance of inheriting the nest is thought to account for the occurrence of unrelated cofoundress helpers in temperate

species, leading to the suggestion that direct fitness (as well as indirect fitness) is important in the early stages of social evolution (Leadbeater et al., 2011). However, nest inheritance may only be important in species where relatedness of cofoundresses, and thus indirect fitness payoffs from helping, are low. In this specific case, the importance of nest inheritance and direct fitness in *P. dominula* may be driven by its temperate environment and resulting seasonal diapause which makes it difficult for females to renest with relatives.

We used latitude to categorise environments into tropical, subtropical, and temperate categories (see Appendix 1). However, these coarse categories overlook variation, e.g. a severe wet/dry season in a tropical region may disrupt nesting cycles to a similar degree to a temperate region (Hunt et al., 1999; González et al., 2002; Gobbi et al., 2006). Likewise, it is hard to generalise on how a subtropical environment (for which data on three species exist; Appendix S1) affects nesting cycle and social dynamics, as the distinctness of a diapause period depends on the local conditions and even the year of study; species in these zones could be useful models for understanding plasticity in nest founding behaviour in response to uncertainty of relatedness (Strassmann, 1981). Further work on the under-studied tropical species (which represent the ancestral conditions), especially including species from the Old World tropics (where *Polistes* is thought to have originated – Carpenter, 1996; Santos et al., 2015), is essential in order to determine the importance of high indirect fitness payoffs from founding nests with relatives as well as putative direct fitness through nest inheritance (Leadbeater et al., 2011) in the origin of group living in societies consisting of many potential reproductive females (Hamilton, 1964).

The lack of a seasonal diapause may provide tropical species with a mechanism by which to improve the chances of cofounding a new nest with relatives. New foundress nests in *P. canadensis* are often formed by groups of females (see data in Miller et al., 2018) from previously abandoned mature post-emergence nest combs (authors *pers. obs.*; Pickering

1980), making family groups (if not the nest structure itself) perennial rather than annual; we provide supporting genetic data for this, as nests usually consist of a mother with her daughters or a group of sisters. In the absence of an overwintering diapause period, opportunities to nest with relatives may be more prevalent for tropical species than for temperate social wasps, as the time lag between leaving the natal nest and founding a new nest is likely short in tropical species (for example, if within-nest gyne dispersal is synchronous or nestmates are recruited nearby). There is little evidence that *Polistes* can distinguish individual kin on the basis of relatedness alone in temperate *Polistes*, although wasps may recognise nestmates or discrete units of kin (i.e. related verses unrelated) (Dani et al., 2004; Gamboa, 2004; Seppä et al., 2012; Leadbeater et al., 2013). Cofounding individuals may be making joining decisions based on advertised signals such as behaviour and nestmate odour, or follow simple rules such as distance from the natal nest (Dapporto et al., 2004; Sumana et al., 2005; Tibbetts & Injaian, 2013; Field & Leadbeater, 2016). This latter case may be an important mechanism in *P. canadensis* for maximizing the chance of nesting with relatives, as nests exist in aggregations of related groups with significant isolation by distance (Lengronne et al., 2012).

Reproductive monopoly was evident at both stages of the nesting cycle. High skew with high relatedness among adults at the nest founding stage generates the conditions for altruism to evolve through indirect fitness benefits. But for helpers to realise these fitness benefits, high skew must be maintained throughout the nesting cycle to the point where new reproductives (gynes and males) are produced. Our findings appear to contrast with those from temperate *Polistes* species, where lower reproductive skew (more egg-layers) is usually found in the foundress period and first brood, and skew then increases as the nesting cycle progresses such that the reproductive brood are the offspring of one original foundress (Field et al., 1998; Reeve et al., 2000; Seppä et al., 2002). High skew in *P. canadensis* is akin to the genetic structure found in the group-founding stage of other independent-founding tropical wasps,

such as *Parischnogaster alternata* (Bolton et al., 2006) and *Liostenogaster flavolineata* (Sumner et al., 2002).

An interesting question raised by our findings is how a reproductive monopoly can be maintained in such large post-emergence nests. The group sizes in our study reached up to 80 adult females (and over 1200 cells); but nests can become much larger, with groups of 200+ wasps observed commonly (Pickering, 1980; Hunt, 2007; authors *pers. obs.*). Dominance hierarchies in *Polistes* are established and maintained primarily through physical aggression and threat displays, such that suppression of reproduction by subordinates is achieved by queen-control (reviewed in Jandt et al., 2014). A queen on a large nest of a tropical species like *P. canadensis* is unlikely to be able to physically dominate all females on the nest; it has been suggested that such queens would be forced to concede reproduction to additional egg-layers in certain 'territories' of the nest (West-Eberhard, 1986). Our analyses indicated that if territorial queens do exist in post-emergence nests, they did not have lasting effects on realised reproductive skew. Instead, our data provide evidence of selection against secondary egg-layers even in these large sexual-producing nests, maintaining high indirect fitness payoffs for subordinate females and incentives for them to refrain (or be policed) from laying male as well as female eggs. However, larger samples of brood per nest would be important to analyse in order to explore the potential payoffs of egg-laying by subordinates in these large nests, as with a sample size of 5–15 brood per nest it is possible that secondary egg-laying events have been missed.

Despite the effective reproductive monopoly in foundress nests, 46% of foundresses possessed mature eggs in their ovary tracts; it has been previously noted that ovarian development alone is an unreliable indicator of reproductive partitioning in *Polistes* (Strassmann et al., 1983; Seppä et al., 2002; Izzo et al., 2010; Cini et al., 2013). Subordinates with developed ovaries may instead be waiting, reproductive-ready, in the wings in the event

that the effective egg-layer leaves, e.g. to drift to another nest (Sumner et al., 2007). Helping whilst queuing to inherit the nest may therefore be a trait explaining altruism for both tropical and temperate species (Leadbeater et al., 2011; Field & Leadbeater, 2016), but the expression of this trait depends on the likelihood of nesting with relatives.

The discovery of occasional multiple mating in this species is surprising: most *Polistes* mate once (reviewed in Strassmann, 2001), though multiple mating has been observed in *Polistes annularis* (spermatheca content: 2 out of 40 females contained an extra mate – Peters et al., 1995) and *Polistes biglumis* (effective paternity 1.20–1.22 in offspring – Seppä et al., 2011). However, it is evident that the effective mating frequency in *P. canadensis* of 1.05 in this study suggests multiple mating is unlikely to have a significant effect on fitness payoffs to workers in nests (as relatedness is high in nests). Multiple mating is a trait of complex sociality, found in species that have evolved beyond the 'monogamy window': in keeping with this idea, significant levels of multiple mating have been detected in the highly eusocial wasps, such as *Vespa crabro* (1.11, Foster et al., 1999), *Dolichovespula sylvestris* (1.15, Foster et al., 2001), and *Dolichovespula arenaria* (1.49, Loope et al., 2015). The low levels of multiple mating in *Polistes* require further investigation: a defining feature of eusocial insects is that they mate during one period in their lives; it has been suggested that this is one trait that sets truly eusocial species apart from cooperative breeders (Boomsma, 2009). If low levels of multiple mating continue to be found in *Polistes,* this may be a reason for *Polistes* to be better described as a cooperative breeder than a eusocial organism (Boomsma, 2009). Future studies should focus on tropical species as they have access to mates at all times (due to nesting asynchrony), to determine whether females mate with multiple males in one period in their life (as in a eusocial organism), or sequentially throughout their life (as in a cooperative breeder). Analysis of how paternity is associated with colony-level demography may help discern this.

We found no evidence of worker production of male eggs in our study. Male brood were only present in the late stage post-emergence nests (as is typical in *Polistes)*, and the dominant mother of the female brood was the mother of all the sampled male brood. Male production by workers has not previously been detected in temperate *Polistes*, and this has been explained by group sizes being small enough for queens to physically suppress worker reproduction (Miyano, 1983; Arévalo et al., 1998; Strassmann et al., 2003). However, in *P. canadensis* queen-control of worker reproduction is likely to be less effective: *P. canadensis* in our study had around twice as many group members (33.6 females per nest) as the temperate and subtropical species that have been studied (e.g. 15–25 females per nest – Arévalo et al., 1998; Strassmann et al., 2003) and so workers may be better able to circumvent physical suppression by the queen. The lack of worker-produced males in this study may reflect the presence of worker policing via differential oophagy of worker-laid eggs by workers (Liebig et al., 2005; Monnin et al., 2009; Cini et al., 2014); analyses of larger nests (e.g. *P. canadensis* nests may have up to 200 wasps, Pickering 1980) may provide a better test of this.

In addition to filling a knowledge gap in our understanding of the genetic structure of *Polistes* nests*,* this study has also generated a new tool kit for genotyping in *Polistes.* The information from genetic analyses has improved over the last few decades as new types of markers with higher level resolution have been developed. Over half of species of *Polistes* in which genetic structure and/or relatedness has been examined using molecular markers come from allozyme data (see Appendix S1). Allozymes are very quick and cheap to use, but they provide very low resolution data that is variable in quality (e.g. estimates of relatedness for *P. canadensis* using allozymes are lower than those obtained from microsatellite and SNP analyses; see Appendix 1). Allozymes were superseded by microsatellites in the late 1990s, and with their superior levels of resolution and reliability, microsatellites have been the marker of choice for genetic structure analyses of social insects with great success. However, it is often challenging to obtain enough highly polymorphic microsatellite loci for new (non-model) organisms and the

process of finding, testing, and optimising microsatellites can be laborious, costly, and unrewarding; the success of cross-species use of loci is variable, with highly polymorphic loci often being of low polymorphism in even close relatives. The burgeoning volume of transcriptomic datasets for a rapidly expanding range of organisms now make it possible to easily identify polymorphic SNPs, and design assays that are polymorphic for a range of related species; SNPs also offer the possibility of high throughput analyses across thousands of loci. In our study we used both SNPs and microsatellites; results from the two datasets were comparable, suggesting that the extra level of resolution offered by the SNPs was not required to discern the genetic structure of these insect societies. However, the utility of these markers across species (see Methods; Southon et al. in prep), now facilitate similar studies in other related (tropical) species without the costly and lengthy process of having to develop microsatellite markers, and provide much-needed analyses on a wider range of tropical *Polistes.*

5. CONCLUSIONS

High relatedness and reproductive skew are likely to be key components for inferring indirect fitness gains for reproductive subordinate cofoundresses in the tropical wasp *P. canadensis*. Our study raises the question of whether direct fitness via nest inheritance is truly an ancestral trait in the emergence of group living in *Polistes*, or a secondary adaptation to diapause in temperate regions. Further studies on species spanning temperate to tropical climates are therefore encouraged to understand fully the influence of environment on social structure and dynamics, and the relative importance of direct and indirect fitness at the origin of group living.

ACKNOWLEDMENTS

We thank the Smithsonian Tropical Research Institute, in particular W.T. Wcislo and D.M. Windsor for facilitating the fieldwork. We thank D. Fabbro and B. Morris for help in the field and

lab respectively. Field research was conducted under ANAM research permit SE/A-55-13. Research was funded by NERC studentships awarded to E.F.B. & R.J.S., and a NERC grant (NE/K011316/1) awarded to S.S. Thanks to A. Cini for comments on a draft of the manuscript, and A.F.G. Bourke, J.R. Bridle, I.C. Cuthill, and E. Leadbeater for comments on related chapters as part of doctoral examinations by E.F.B and R.J.S. We thank the editor and six anonymous reviewers for their contributions to the manuscript.

REFERENCES

Arévalo, E., Strassmann, J.E., & Queller, D.C. (1998). Conflicts of interest in social insects: male production in two species of *Polistes*. *Evolution*, *52*(3), 797–805.

Armitage, K.B. (2007). Evolution of Sociality in Marmots: It Begins with Hibernation. In J.O. Wolff & P.W. Sherman (Eds.) *Rodent Societies: An Ecological & Evolutionary Perspective* (pp. 356–367). Chicago, IL: University of Chicago Press.

Bolton, A., Sumner, S., Shreeves, G., Casiraghi, M., & Field, J. (2006). Colony genetic structure in a facultatively eusocial hover wasp. *Behavioral Ecology*, *17*(6), 873–880.

Boomsma, J.J. (2007). Kin selection versus sexual selection: why the ends do not meet. *Current Biology*, *17*(16), R673–R683.

Boomsma, J.J. (2009). Lifetime monogamy and the evolution of eusociality. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *364*(1533), 3191–3207.

Bourke, A.F.G. (2014). Hamilton's rule and the causes of social evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1642), 1–10.

Cant, M.A., & Johnstone, R.A. (2008). Reproductive conflict and separation of reproductive generations in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(14), 5332–5336.

Carpenter, J.M. (1996). Phylogeny and biogeography of *Polistes*. In S. Turillazzi & M.J. West-Eberhard (Eds.), *Natural History and Evolution of Paper-Wasps* (pp. 18–57). Oxford: Oxford University Press.

Cini, A., Meconcelli, S., & Cervo, R. (2013). Ovarian indexes as indicators of reproductive investment and egg-laying activity in social insects: a comparison among methods. *Insectes Sociaux*, *60*(3), 393–402.

Cini, A., Nieri, R., Dapporto, L., Monnin, T., & Cervo, R. (2014). Almost royal: incomplete suppression of host worker ovarian development by a social parasite wasp. *Behavioral Ecology and Sociobiology*, *68*(3), 467–475.

Clutton-Brock, T. (2009). Cooperation between non-kin in animal societies. *Nature*, *462*(7269), 51–57.

Cockburn, A., & Russell, A.F. (2011). Cooperative breeding: a question of climate? *Current Biology*, *21*(5), R195–R197.

Cronin, A.L., Bridge, C., & Field, J. (2011). Climatic correlates of temporal demographic variation in the tropical hover wasp *Liostenogaster flavolineata. Insectes Sociaux*, *56*(1), 23–29.

Danforth, B.N., & Eickwort, G.C. (1997). The evolution of social behavior in the augochlorine sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera. In J.C. Choe & B.J. Crespi (Eds.), *The Evolution of Social Behaviour in Insects and Arachnids* (pp. 270–292). Cambridge: Cambridge University Press.

Dani, F.R., Foster, K.R., Zacchi, F., Seppä, P., Massolo, A., Carelli, A., Arévalo, E., Queller., D.C., Strassmann, J.E., Turillazzi, S. (2004). Can cuticular lipids provide sufficient information for within-colony nepotism in wasps? *Proceedings of the Royal Society of London B: Biological Sciences*, *271*(1540), 745–753.

Dapporto, L., Pansolli, C., & Turillazzi, S. (2004). Hibernation clustering and its consequences for associative nest foundation in *Polistes dominulus* (Hymenoptera Vespidae). *Behavioral Ecology and Sociobiology*, *56*(4), 315–321.

Denlinger, D.L. (1986). Dormancy in tropical insects. *Annual Review of Entomology*, *31*(1), 239–264.

Ferreira, P. G., Patalano, S., Chauhan, R., Ffrench-Constant, R., Gabaldon, T., Guigo, R., & Sumner, S. (2013). Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes. *Genome Biology*, *14*(2), R20.

Field, J., Solís, C.R., Queller, D.C., & Strassmann, J.E. (1998). Social and genetic structure of paper wasp cofoundress nests: tests of reproductive skew models. *The American Naturalist*, *151*(6), 545–563.

Field, J., Paxton, R.J., Soro, A., & Bridge, C. (2010). Cryptic plasticity underlines a major evolutionary transition. *Current Biology*, *20*(22), 2028–2031.

Field, J., & Leadbeater, E. (2016). Cooperation between non-relatives in a primitively eusocial paper wasp *Polistes dominula*. *Philosophical Transactions of The Royal Society B: Biological Sciences*, *371*(1687), 1–6.

Foster, K.R., Seppä, P., Ratnieks, F.L., & Thorén, P.A. (1999). Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioral Ecology and Sociobiology*, *46*(4), 252–257.

Foster, K.R., Ratnieks, F.L.W., Gyllenstrand, N., Thorén, P.A. (2001). Colony kin structure and male production in *Dolichovespula* wasps. *Molecular Ecology*, *10*(4), 1003–1010.

Fucini, S., Di Bona, V., Mola, F., Piccaluga, C., & Lorenzi, M.C. (2009). Social wasps without workers: geographic variation of caste expression in the paper wasp *Polistes biglumis*. *Insectes Sociaux*, *56*(4), 347–358.

Gamboa, G.J. (2004). Kin recognition in eusocial wasps. *Annales Zoologici Fennici*, *41*(6), 789–808.

Gobbi, N., Noll, F.B., & Penna, M.A.H. (2006). “Winter” aggregations, colony cycle, and seasonal phenotypic change in the paper wasp *Polistes versicolor* in subtropical Brazil. *Naturwissenschaften*, *93*(10), 487–494.

González, J.A., Nascimento, F.S., & Gayubo, S.F. (2002). Observations on the winter aggregates of two polistine paper wasps (Hymenoptera Vespidae Polistinae). *Tropical Zoology*, *15*(1), 1–4.

Hamilton, W.D. (1964). The genetical evolution of social behaviour, I & II. *Journal of Theoretical Biology*, *7*, 1–52.

Hunt, J.H., Brodie, R.J., Carithers, T.P., Goldstein, & P.Z., Janzen, D.H. (1999). Dry Season Migration by Costa Rican Lowland Paper Wasps to High Elevation Cold Dormancy Sites. *Biotropica*, *31*(1), 192–196.

Hunt, J.H. (2007). The Evolution of Social Wasps. New York, NY: Oxford University Press.

Izzo, A., Wells, M., Huang, Z., Tibbetts, E. (2010). Cuticular hydrocarbons correlate with fertility not dominance, in a paper wasp, *Polistes dominulus*. *Behavioral Ecology and Sociobiology*, *64*(5), 857–864.

Jandt, J.M., Tibbetts, E.A., & Toth, A.L. (2014). *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. *Insectes Sociaux*, *61*(1), 11–27.

Jeanne, R.L. (1979). Construction and utilization of multiple combs in *Polistes canadensis* in relation to the biology of a predaceous moth. *Behavioral Ecology and Sociobiology*, *4*(3), 293–310.

Jeanne, R.L. (1980). Evolution of social behavior in the Vespidae. *Annual Review of Entomology*, *25*, 371–396.

Jetz, W., & Rubenstein, D.R. (2011). Environmental uncertainty and the global biogeography of cooperative breeding in birds. *Current Biology*, *21*(1), 72–78.

Jombart, T. (2008). *adegenet*: a R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405.

Jombart, T., & Ahmed, I. (2011). *adegenet 1.3-1*: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071.

Jones, O.R., & Wang, J. (2010). COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources*, *10*(3), 551–555.

Keller, L., & Reeve, H.K. (1999). Dynamics of Conflict within Insect Societies. In L. Keller (Ed.), *Levels of Selection in Evolution* (pp. 153–175). Princeton, NJ: Princeton University Press.

Keller, L. (2003). Behavioral plasticity: levels of sociality in bees. *Current Biology*, *13*(16), R644–R645

Kokko, H., & Johnstone, R.A. (1999). Social queuing in animal societies: a dynamic model of reproductive skew. *Proceedings of the Royal Society of London B: Biological Sciences*, *266*(1419), 571–578.

Koštál, V. (2006). Eco-physiological phases of insect diapause. *Journal of Insect Physiology*, *52*(2), 113–127.

Krause, J., & Ruxton, G.D. (2002). Living in Groups. Oxford: Oxford University Press.

Leadbeater, E., Carruthers, J. M., Green, J. P., van Heusden, J. & Field, J.P. (2010) Unrelated helpers in a primitively eusocial wasp: is helping tailored towards direct fitness? *PLoS ONE*, *5(8),* e11997

Leadbeater, E., Carruthers, J.M., Green, J.P., Rooser, N.S., & Field, J. (2011). Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science*, *333*(6044), 874–876.

Leadbeater, E., Dapporto, L., Turillazzi, S., & Field, J. (2013). Available kin recognition cues may explain why wasp behavior reflects relatedness to nest mates. *Behavioral Ecology*, *25*(2), 344–351.

Lengronne, T., Leadbeater, E., Patalano, S., Dreier, S., Field, J., Sumner, S., & Keller, L. (2012). Little effect of seasonal constraints on population genetic structure in eusocial paper wasps. *Ecology and Evolution*, *2*(10), 2615–2624.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map (SAM) format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079.

Li, H. (2011). A statistical framework for SNP calling, mutation discovery, nest mapping and population genetical parameter estimation from sequencing data. *Bioinformatics*, *27*(21), 2987–2993.

Liebert, A.E., Johnson, R.N., Switz, G.T., & Starks, P.T. (2004). Triploid females and diploid males: underreported phenomena in *Polistes* wasps? *Insectes Sociaux*, *51*(3), 205–211.

Liebert, A.E., Nonacs, P., & Wayne, R.K. (2005). Solitary nesting and reproductive success in the paper wasp *Polistes aurifer*. *Behavioral Ecology and Sociobiology*, *57*(5), 445–456.

Liebig, J., Monnin, T., & Turillazzi, S. (2005). Direct assessment of queen quality and lack of worker suppression in a paper wasp. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*(1570), 1339–1344.

Loope, K.J. (2015). Queen Killing Is Linked to High Worker-Worker Relatedness in a Social Wasp. *Current Biology*, *25*(22), 2976–2979.

Lynch, M., & Ritland, K. (1999). Estimation of pairwise relatedness with molecular markers. *Genetics*, *152*(4), 1753–1766.

McCracken, G.F., & Wilkinson, G.S. (2000). Bat Mating Systems. In E.G. Crichton & P.H. Krutzsch (Eds.), *Reproductive Biology of Bats* (pp. 321–362). London: Academic Press.

Metcalf, R.A., & Whitt, G.S. (1977). Intra-nest relatedness in the social wasp *Polistes metricus*. *Behavioral Ecology and Sociobiology*, *2*(4), 339–351.

Miller, S.E., Bluher, S.E., Bell, E., Cini, A., da Silva, R.C., de Souza, A.R., Gandia, K.M., Jandt, J., Loope, K., Prato, A., Pruitt, J.N., Rankin, D., Rankin, E., Southon, R.J., Uy, F.M.K., Weiner, S., Wright, C.M., Downing, H., Gadagkar, R., Lorenzi, M.C., Rusina, L., Sumner, S., Tibbetts, E.A., Toth, A., Sheehan, M.J. (2018). WASPnest: a worldwide assessment of social Polistine nesting behavior. *Ecology*, *99*(10), 2405.

Miyano, S. (1983). Number of offspring and seasonal changes of their body weight in a paperwasp, *Polistes chinensis antennalis* Pérez (Hymenoptera: Vespidae), with reference to male production. *Researches on Population Ecology*, *25*(1), 198–209.

Monnin, T., Cini, A., Lecat, V., Fédérici., P., & Doums, C. (2009). No actual conflict over colony inheritance despite high potential conflict in the social wasp *Polistes dominulus. Proceedings of the Royal Society B: Biological Sciences*, *276*(1662), 1593–1601.

Montero-Pau, J., Gómez, A., & Muñoz, J. (2008). Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnology and Oceanography: Methods*, *6*(6), 218–222.

Niehuis O, Gibson JD, Rosenberg MS, Pannebakker BA, Koevoets T, et al. (2010) Recombination and its impact on the genome of the haplodiploid parasitoid wasp *Nasonia*. *PLoS ONE, 5(*1*),* e8597.

Nielsen, R., Tarpy, D.R., & Reeve, H.K. (2003). Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, *12*(11), 3157–3164.

Nonacs, P. (2000). Measuring and using skew in the study of social behavior and evolution. *The American Naturalist*, *156*(6), 577–589.

Nonacs, P. (2003). Measuring the reliability of skew indices: is there one best index? *Animal Behaviour*, *65*(3), 615–627.

O'Donnell, S., & Joyce, F.J. (2001). Seasonality and colony composition in a montane tropical eusocial wasp. *Biotropica*, *33*(4), 727–732.

Paradis, E. (2010). pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics*, *26*(3), 419–420.

Patalano, S., Vlasova, A., Wyatt, C., Ewels, P., Camara, F., Ferreira, P.G., Asher, C.L., Jurkowski, T.P., Segonds-Pichon, A., Bachman, M., González-Navarrete, I., Minoche, A.E., Krueger, F., Lowy, E., Marcet-Houben, M., Luis Rodriguez-Ales, J., Nascimento, F.S., Balasubramanian, S., Gabaldon, T., Tarver, J.E., Andrews, S., Himmelbauer, H., Hughes, W.O.H., Guigó, R., Reik, W., & Sumner, S. (2015). Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(45), 13970–13975.

Peters, J.M., Queller, D.C., Strassmann, J.E., & Solís, C.R. (1995). Maternity assignment and queen replacement in a social wasp. *Proceedings of the Royal Society B: Biological Sciences*, *260*(1357), 7–12.

Pickering, J. (1980). Sex ratio, social behavior and ecology in *Polistes* (Hymenoptera, Vespidae), *Pachysomoides* (Hymenoptera, Ichneumonidae) and *Plasmodium* (Protozoa, Haemosporida). Cambridge, MA: Harvard University.

Queller, D.C., Peters, J.M., Solís, C.R., & Strassmann, J.E. (1997). Control of reproduction in social insect colonies: individual and collective relatedness preferences in the paper wasp, *Polistes annularis*. *Behavioral Ecology and Sociobiology*, *40*(1), 3–16.

Queller, D.C., & Strassmann, J.E. (1998). Kin selection and social insects. *BioScience*, *48*(3), 165–175.

Queller, D.C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M.T., Santorelli, L.A., & Strassmann, J.E. (2000). Unrelated helpers in a social insect. *Nature*, *405*(6788), 784–787.

R Core Team. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Reeve, H.K. (1991). *Polistes*. In K.G. Ross & R.W. Matthews (Eds.), *The Social Biology of Wasps* (pp. 99–148). Ithaca, NY: Cornell University Press.

Reeve, H.K., Starks, P.T., Peters, J.M., & Nonacs, P. (2000). Genetic support for the evolutionary theory of reproductive transactions in social wasps. *Proceedings of the Royal Society of London B: Biological Sciences*, *267*(1438), 75–79.

Reeve, H.K., & Keller, L. (2001). Test of reproductive-skew models in social insects. *Annual Review of Entomology*, *46*(1), 347–385.

Richards, M.H. (2000). Evidences for geographic variation in colony social organization in an obligately social sweat bee, *Lasioglossum malachurum* Kirby (Hymenoptera; Halictidae). *Canadian Journal of Zoology*, *78*(7), 1259–1266.

Robinson, J.T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E.S., Getz, G., & Mesirov, J.P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26.

Rousset, F. (2008). GENEPOP'077: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106.

Santos, B.F., Payne, A., Pickett, K.M., & Carpenter, J.M. (2015). Phylogeny and historical biogeography of the paper wasp genus *Polistes* (Hymenoptera: Vespidae): implications for the overwintering hypothesis of social evolution. *Cladistics*, *31*(5), 535–549.

Semagn, K., Babu, R., Hearne, S., & Olsen, M. (2014). Simple nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement. *Molecular Breeding*, *33*(1), 1–14.

Seppä, P., Queller, D.C., & Strassmann, J.E. (2002). Reproduction in foundress nests of the social wasp *Polistes carolina*: conventions, competition, and skew. *Behavioral Ecology*, *13*(4), 531–542.

Seppä, P., Fogelqvist, J., Gyllenstrand, N., & Lorenzi, M.C. (2011). Colony kin structure and breeding patterns in the social wasp, *Polistes biglumis*. *Insectes sociaux*, *58*(3), 345–355.

Seppä, P., Queller, D.C., & Strassmann, J.E. (2012). Why Wasp Foundresses Change Nests: Relatedness, Dominance, and Nest Quality. *PLoS ONE*, *7*(9), e45386.

Sheehan, M.J., Botero, C.A., Hendry, T.A., Sedio, B.E., Jandt, J.M., Weiner, S., Toth, A.L., & Tibbetts, E.A. (2015). Different axes of environmental variation explain the presence vs. extent of cooperative nest founding associations in *Polistes* paper wasps. *Ecology Letters*, *18*(10), 1057–1067.

Shokralla, S., Singer, G.A., & Hajibabaei, M. (2010). Direct PCR amplification and sequencing of specimens' DNA from preservative ethanol. *Biotechniques*, *48*(3), 305–306.

Starks, P.T. (2003). Natal nest discrimination in the paper wasp, *Polistes dominulus*. *Annales Zoologici Fennici*, 40, 53–60.

Strassmann, J.E. (1981). Evolutionary implications of early male and satellite nest production in *Polistes exclamans* colony cycles. *Behavioral Ecology and Sociobiology*, *8*(1), 55–64.

Strassmann, J.E. (1983). Nest fidelity and group size among foundresses of *Polistes annularis* (Hymenoptera: Vespidae). *Journal of the Kansas Entomological Society*, *56*(4), 621–634.

Strassmann, J.E., Hughes, C.R., Queller, D.C., Turillazzi, S., Cervo, R., Davis, S.K., & Goodnight, K.F. (1989). Genetic relatedness in primitively eusocial wasps. *Nature*, *342*(6247), 268–270.

Strassmann, J.E. (2001). The rarity of multiple mating by females in the social Hymenoptera. *Insectes Sociaux*, *48*(1), 1–13.

Strassmann, J.E., Nguyen, J.S., Arévalo, E., Cervo, R., Zacchi, F., Turillazzi, S., & Queller, D.C. (2003). Worker interests and male production in *Polistes gallicus*, a Mediterranean social wasp. *Journal of Evolutionary Biology*, *16*(2), 254–259.

Sumana, A., Liebert, A.E., Berry, A.S., Switz, G.T., Orians, C.M., & Starks, P.T. (2005). Nest hydrocarbons as cues for philopatry in a paper wasp. *Ethology*, *111*(5), 469–477.

Sumner, S., Casiraghi, M., Foster, W., & Field, J. (2002). High reproductive skew in tropical hover wasps. *Proceeding of the Royal Society of London B: Biological Sciences*, *269*(1487), 179–186.

Sumner, S., Pereboom, J.J., & Jordan, W.C. (2006). Differential gene expression and phenotypic plasticity in behavioural castes of the primitively eusocial wasp, *Polistes canadensis*. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*(1582), 19–26.

Sumner, S., Lucas, E., Barker, J., Isaac, N. (2007). Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. *Current Biology*, *17*(2), 140–145.

Sumner, S., Kelstrup, H., & Fanelli, D. (2010). Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*(1688), 1721–1728.

Thorvaldsdóttir, H., Robinson, J.T., & Mesirov, J.P. (2013). Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. *Briefings in Bioinformatics*, *14*(2), 178–192.

Tibbetts, E.A., & Injaian, A., (2013). Preferential phenotypic association linked with cooperation in paper wasps. *Journal of Evolutionary Biology, 26*(11), 2350–2358.

Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A.A., Walker, J.A., & Warman, M.L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *Biotechniques*, *29*(1), 52–54.

Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. *Genetics*, *160*(3), 1203–1215.

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*(1), 141–145.

West, S.A., Pen, I., & Griffin, A.S. (2002). Cooperation and competition between relatives. *Science*, *296*(5565), 72–75.

West, S.A., Griffin, A.S., & Gardner, A. (2007). Evolutionary explanations for cooperation. *Current Biology*, *17*(16), R661–R672.

West-Eberhard, M.J. (1969). The Social Biology of Polistine Wasps. *Miscellaneous Publications Museum of Zoology, University of Michigan*, *140*, 1–101.

West-Eberhard, M.J. (1986). Dominance relations in *Polistes canadensis* (L.), a tropical social wasp. *Monitore Zoologico Italiano-Italian Journal of Zoology*, *20*(3), 263–281.

Whitfield, C.W., Behura, S.K., Berlocher, S.H., Clark, A.G., Johnston, J.S., Sheppard, W. S., Smith, D.R., Suarez, A.V., Weaver, D., & Tsutsui, N.D. (2006). Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science*, *314*(5799), 642–645.

Wilson, E.O. (1971). The Insect Societies. Cambridge, MA: Belknap Press of Harvard University Press.

Wittemyer, G., Getz, W.M., Vollrath, F., & Douglas-Hamilton, I. (2007). Social dominance, seasonal movements, and spatial segregation in African elephants: a contribution to conservation behavior. *Behavioral Ecology and Sociobiology*, *61*, 1919–1931.

Zanette, L.R.S., & Field, J. (2008). Genetic relatedness in early nests of *Polistes dominula*: from related to unrelated helpers. *Molecular Ecology*, *17*(11), 2590–2597.

DATA ACCESSIBILITY

Supplementary datasets (including SNP and microsatellite genotypes) provided. SNP reads have been deposited at NCBI dbSNP Short Genetic Variations database, batch number 1062865 and can be accessed at: https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ss.cgi?subsnp_id=3023075125

SUPPLEMENTARY DATASETS

Appendix S1. Literature review of *Polistes* genus studies.

Appendix S2. Nesting group characteristics for foundress and post-emergence nests used in the study.

Appendix S3. Full database for 1791 SNPs identified for *P. canadensis* and *P. lanio*. Includes Accessions numbers for NCBI's dbSNP.

Appendix S4. 120 SNPs for validation. Position, count, coverage, and frequency for the 120 SNP loci discovered in *P. canadensis* and *P. lanio*.

Appendix S5. Genotyped sample summary. Sample sizes of *P. canadensis* from across the nesting cycle and life-stages used in this study.

Appendix S6. SNP genotypes (valid 69 SNPs).

Appendix S7. Pairwise relatedness calculated in COANCESTRY 1.0.1.8 (Wang, 2011), reporting the Wang (2002) and Lynch & Ritland (1999) relatedness estimation.

Appendix S8. Ovary dissections for genotyped adults. Ovary grades (adapted from Gobbi et al., 2006): A = small filamentous ovarioles lacking oocytes; B = small ovarioles with slightly developed oocytes; C = large ovarioles with few developed oocytes at the base of tract; D = large ovaries with multiple fully developed oocytes; and E = large ovaries with visible regression. Mature egg: if longer than 2 mm (Sumner et al., 2006).

Appendix S9. Preliminary COLONY 2.0.6.5 (Jones & Wang, 2010) model for determining potential mothers in foundress colonies among adult females (medium run length) (SNP dataset).

Appendix S10. COLONY 2.0.6.5 models for determining pedigree structures (via preliminary model for foundresses, Appendix S9).

Appendix S11. Alternative COLONY 2.0.6.5 (Jones & Wang, 2010) models for adult female foundresses: potential polyandry ran at genotyping error rates of 0%, 1%, 10%, and forced monandry ran at an error rate of 1% (medium run length) (SNP dataset).

Appendix S12. *B* index per nest calculated in SKEW CALCULATOR 2003 (© Peter Nonacs).

Appendix S13. Type of social structure among female cofoundresses in *P. canadensis* as determined by relatedness estimates and sib-group assignments: 'Matrilineal' type refers to a mother and her daughters; 'Sister' type refers to one or more group of sisters; 'Mixed' type refers to a mix of relationships. Foundress nest relatedness calculated using the Wang estimate (Wang, 2002) in COANCESTRY 1.0.1.8 (Wang, 2011); Maternity assignment constructed in COLONY 2.0.6.5 (Jones & Wang, 2010); and reproductive skew represented as the B index (Nonacs, 2000), calculated in SKEW CALCULATOR 2003 © Peter Nonacs. Nest ID #1–11 SNP dataset, ID #12–19 microsatellite.

Appendix S14. Adult and brood relatedness on post-emergence nests. Relatedness was calculated using the Wang estimate (Wang, 2002) in COANCESTRY 1.0.1.8 (Wang, 2011);

Maternity assignment constructed in COLONY 2.0.6.5 (Jones & Wang, 2010); and reproductive skew represented as the B index (Nonacs, 2000), calculated in SKEW CALCULATOR 2003 © Peter Nonacs. Nest ID #1–10 SNP dataset, ID #11–34 microsatellite (* in reproductive skew marks nests with multiple matrilines).

Appendix S15. The effective mating frequency of females ke3 (Nielsen et al., 2003) across all matrilines (with more than one offspring).

Appendix S16. Microsatellite genotypes (including genotypes from Lengronne et al., 2002).

AUTHOR CONTRIBUTIONS

R.J.S., E.F.B., A.N.R., & S.S. designed the study. P.G., C.W., & R.J.S. developed the SNP markers. E.F.B. & R.J.S. conducted the fieldwork, genotyping, and additional lab work. R.J.S., A.N.R., & S.S. wrote the draft, with contributions from all authors.

FIGURE LEGENDS

Figure 1. Nesting cycle with descriptions, adapted from Pickering (1980), of *P. canadensis* in tropical Panama (including predictions for this study).

Figure 2. Pairwise relatedness (Wang estimate – Wang, 2002) distribution of adult cofoundresses within groups (pairwise relatedness to 1 d.p.).

Figure 3. (A) Percentage of adult females in foundress nests assigned to a direct matriline within each of 11 nests (columns). Three types of family structure were identified: Matrilineal (mother founding a new nest with daughters), Sisters (single or two sib-groups of sisters founding a new nest), and Mixed (mother founding a new nest with daughters, but where a daughter is the primary egg-layer). Each bar represents a unique sib-group, unless indicated by a number; the numbers correspond to the same sib-groups across multiple nests and/or

adults and brood within and across Figure 3A and 3B. Filled-grey bars indicate sib-groups which the mother was present on the nest.

Figure 4. (A) Number of subordinate cofoundresses (those who were not identified as mothers of the genotyped brood) with/without a mature egg (>2 mm in length). (B) Percentage of foundress nests with ovaries graded: A = small filamentous ovarioles lacking oocytes; B = small ovarioles with slightly developed oocytes; C = large ovarioles with few developed oocytes at the base of tract; D = large ovaries with multiple fully developed oocytes; and E = large ovaries with visible signs of regression (adapted from Gobbi et al., 2006). No grade E (large ovaries showing visible regression) were discovered. The ratio number of dominants (egg-layers identified from genotypic analyses) to subordinate cofoundress in the nest are indicated to the right of the columns; * indicates a female who was identified on two nests (i.e. a drifter) and thus represents a duplicate across the two nests.

Figure 1

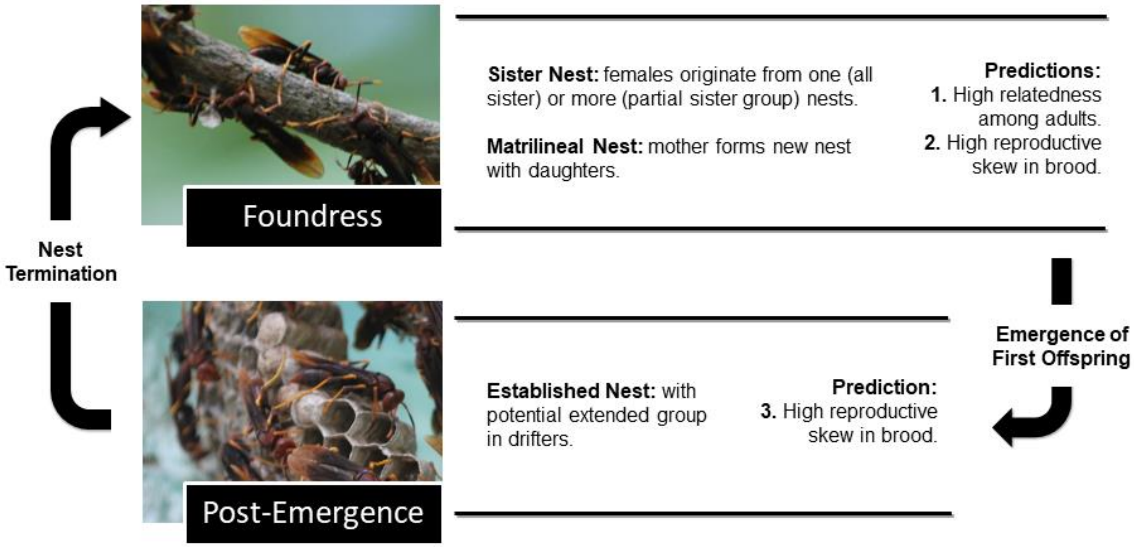

Foundress
Sister Nest: females originate from one (all sister) or more (partial sister group) nests.
Matrilineal Nest: mother forms new nest with daughters.
Predictions:
1. High relatedness among adults.
2. High reproductive skew in brood.
Emergence of First Offspring
Post-Emergence
Established Nest: with potential extended group in drifters.
Prediction:
3. High reproductive skew in brood.
Nest Termination

Figure 2

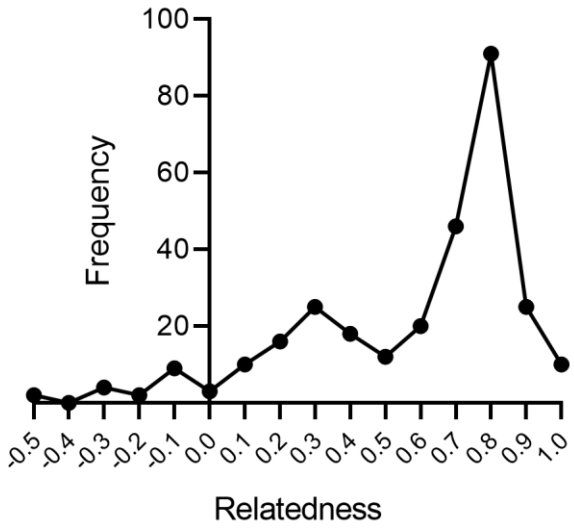

100
80
60
40
20
Frequency
-0.5
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Relatedness

Figure 3a

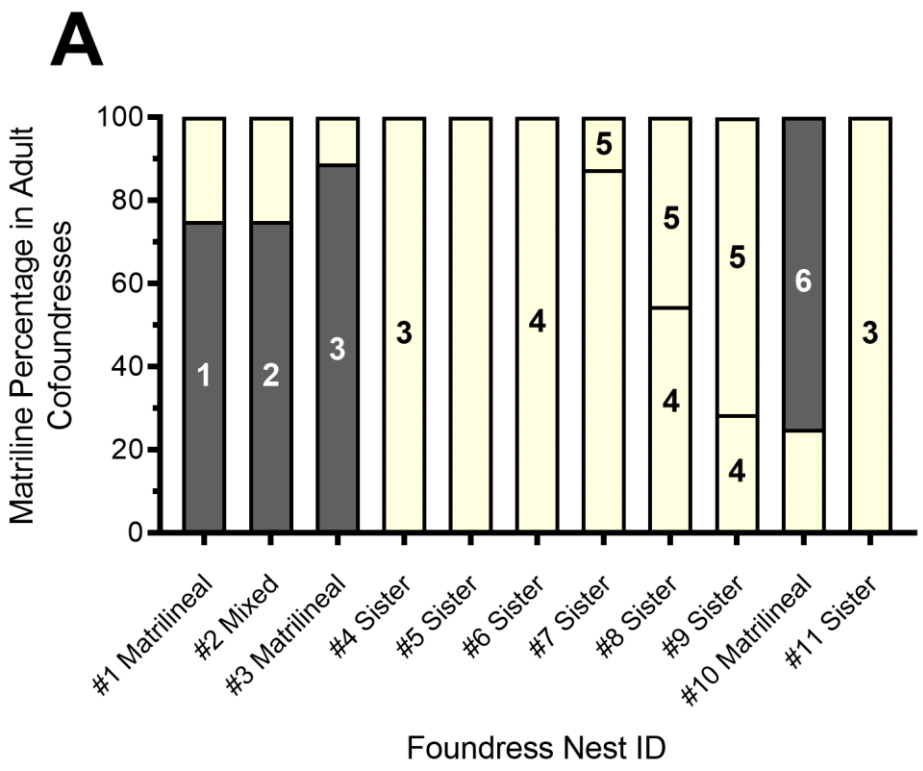

A
Matriline Percentage in Adult Cofoundresses
100
80
60
40
20
0
1
2
3
3
4
5
5
4
5
4
6
3
#1 Matrilineal
#2 Mixed
#3 Matrilineal
#4 Sister
#5 Sister
#6 Sister
#7 Sister
#8 Sister
#9 Sister
#10 Matrilineal
#11 Sister
Foundress Nest ID

Figure 3b

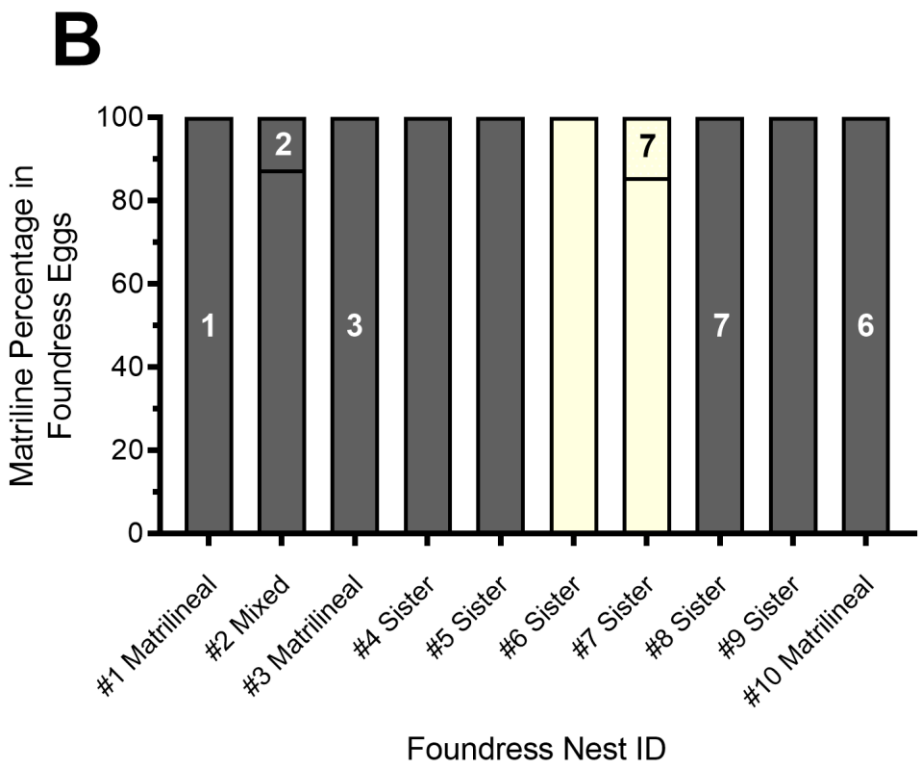

B
Matriline Percentage in Foundress Eggs
100
80
60
40
20
0
1
2
3
7
7
6
#1 Matrilineal
#2 Mixed
#3 Matrilineal
#4 Sister
#5 Sister
#6 Sister
#7 Sister
#8 Sister
#9 Sister
#10 Matrilineal
Foundress Nest ID

Figure 4a

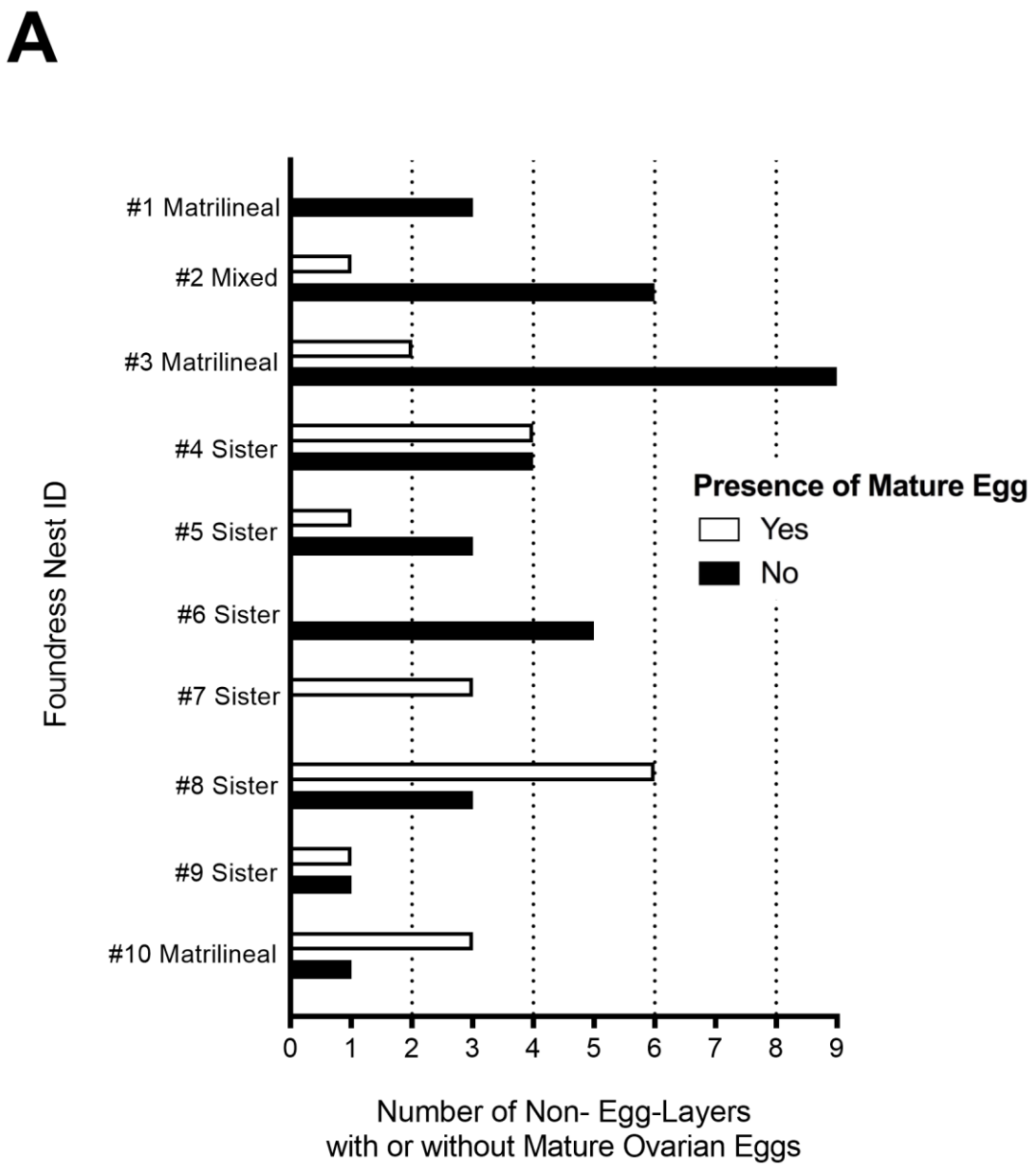
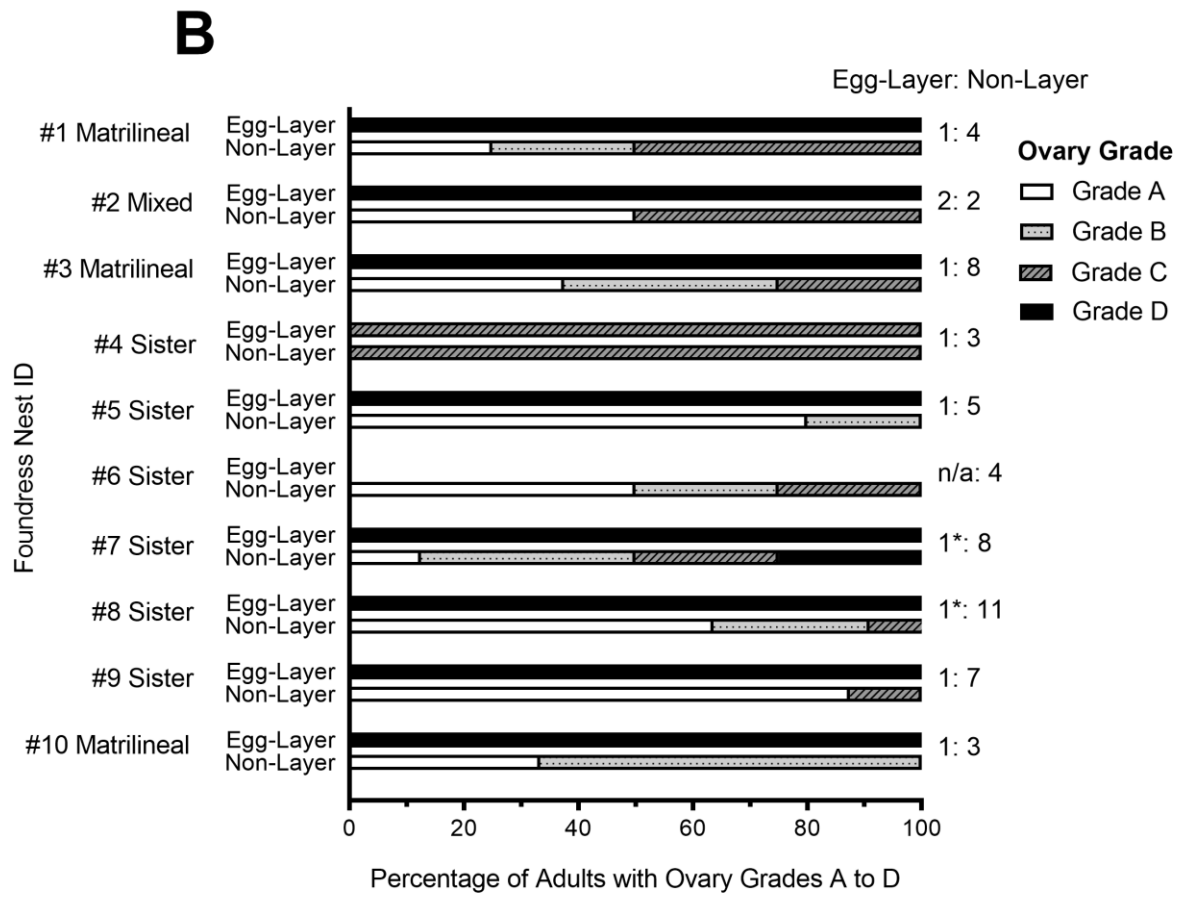

A
#1 Matrilineal
#2 Mixed
#3 Matrilineal
#4 Sister
#5 Sister
#6 Sister
#7 Sister
#8 Sister
#9 Sister
#10 Matrilineal
Foundress Nest ID
Presence of Mature Egg
Yes
No
0 1 2 3 4 5 6 7 8 9
Number of Non- Egg-Layers
with or without Mature Ovarian Eggs

Figure 4b


B
Egg-Layer: Non-Layer
#1 Matrilineal
#2 Mixed
#3 Matrilineal
#4 Sister
#5 Sister
#6 Sister
#7 Sister
#8 Sister
#9 Sister
#10 Matrilineal
Egg-Layer
Non-Layer
1: 4
2: 2
1: 8
1: 3
1: 5
n/a: 4
1*: 8
1*: 11
1: 7
1: 3
Foundress Nest ID
0
20
40
60
80
100
Percentage of Adults with Ovary Grades A to D
Ovary Grade
Grade A
Grade B
Grade C
Grade D