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21 **High reproductive skew in the Neotropical paper**

22 **wasp *Polistes lanio***

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50 **Abstract**

51 Reproductive conflicts are expected in societies where nonbreeding helpers retain the ability to produce
52 offspring. Despite potential competition from reproductively capable nestmates in social wasps, egg
53 laying tends to be monopolised by a single or relatively few queens. Genetic studies on reproductive
54 partitioning in *Polistes* paper wasps suggest high reproductive skew in the genus. Conflict is thought to
55 be minimal due to nestmate relatedness or the possibility of inheriting a reproductive monopoly on a
56 nest; consequently, there are inclusive fitness opportunities for nonreproductive helpers. However, most
57 studies are limited to temperate wasp species. Given the cosmopolitan distribution of *Polistes*, genetic
58 data on group conflicts are required for a broader range of tropical species to determine whether these
59 trends apply across climatic zones. We examined female reproductive skew in the Neotropical paper
60 wasp *Polistes lanio*, genotyping a selection of adults and pupae from established post-emergence nests
61 using single-nucleotide polymorphisms (SNPs). SNP-based pedigree analyses indicate a reproductive
62 monopoly held by a single queen, with queen replacement from natal nestmates and evidence of
63 possible multiple mating. Relatedness between pupal offspring was high ($r = 0.71$). It is likely that high
64 reproductive skew among females is a founding trait of *Polistes* societies, conserved among species
65 that have spread into new environments from Indomalayan origins.

66

67 **Introduction**

68 Societies with many potential breeders must resolve within-group reproductive conflict over who
69 produces young (West et al., 2002; Clutton-Brock, 2009). Reproductive skew measures the outcome
70 of such conflict. High reproductive skew is representative of societies in which reproduction is
71 monopolised by a few breeding individuals, whilst low skew is typical of egalitarian societies where
72 reproduction is more equally shared (Reeve & Keller, 2001; Ratnieks et al., 2006). When reproduction
73 in a group is limited to a few individuals, there must be a payoff to nonreproductives to continue
74 cooperating (Hamilton, 1964; Aureli & de Waal, 2000). Revealing social contracts between group
75 members allows the detection of ancestral and derived novel traits within a clade of species.

76

77 A reproductive division of labour defines most social insect societies, with a reproducing queen (or
78 sometimes multiple queens) and nonreproductive helpers (workers) who raise the queen's brood. In
79 the eusocial Hymenoptera, helpers can be sterile and unable to reproduce (e.g. honeybees and many

80 species of ants), or totipotent and reproductively suppressed (e.g. *Polistes* paper wasps). The genus
81 *Polistes* includes some of the best-studied species of social wasps, which have proven popular models
82 for testing theories on reproductive partitioning because all group members are reproductively totipotent
83 (to a degree), whether they are functional queens or helping workers (West-Eberhard, 1969; West-
84 Eberhard, 1996; Bell & Sumner, 2013; Jandt et al., 2014). Though *Polistes* societies contain many
85 potential reproductives, conflict is expected to be reduced by relatedness among nestmates or the value
86 of inheriting a nest with high reproductive skew, respectively, providing indirect and future direct fitness
87 opportunities to nonreproductive helpers (Queller & Strassmann, 1998; Leadbeater et al., 2011).
88 Despite *Polistes* being geographically widespread, most genetic studies to date have focused on
89 temperate species (Queller et al., 1997; Reeve et al., 2000; Seppä et al., 2002; Liebert et al., 2005;
90 Leadbeater et al., 2011; Southon et al., 2019). Key differences in the ecologies of temperate and tropical
91 species may alter reproductive opportunities for queens and helpers; for example, tropical species
92 typically have perennial nesting cycles and lack stringent winter diapause, allowing continuous mating
93 opportunities and larger nest structures in terms of both size and membership relative to temperate
94 species (West-Eberhard, 1969; Pickering, 1980; O'Donnell & Joyce, 2001). We lack a broad literature
95 on fine-scale genetic analyses of family structures and reproductive skew among group members of
96 tropical *Polistes* species (Southon et al., 2019). Such genetic-structure data are essential for a proper
97 understanding of potential variation in social organisation, and in how conflicts over reproduction are
98 resolved in the representative early stages of social evolution in a cosmopolitan distributed genus, such
99 as *Polistes* (Bourke, 2014).

100

101 We genotyped queens, foragers (helpers) and offspring of the Neotropical paper wasp *Polistes lanio* to
102 determine shared parentage and relatedness within established post-emergence nesting groups. *P.*
103 *lanio* foundresses are pleometrotic; nests are formed by several reproductively capable females who
104 initially compete for dominance (Rusina et al., 2007). Moreover, helpers in this species, like other
105 tropical *Polistes*, appear to retain reproductive potential (e.g. West-Eberhard, 1969; Sumner et al.,
106 2010); this is because nests can be founded at any time of year, and colonies are asynchronous such
107 that males are always available for mating. As such, most female wasps have the opportunity to become
108 reproductives. Following trends found in multiple species of temperate *Polistes* and in the tropical sister

109 species *Polistes canadensis* (Southon et al., 2019), we predicted that *P. lanio* would exhibit high female
110 reproductive skew indicating queen monopolisation of offspring.

111

112 **Materials and methods**

113 Ten post-emergence nests were collected from two sites 5.6 km apart in northern Trinidad, Trinidad &
114 Tobago, June 2013 (five nests from Verdant Vale – 10°41'5.44"N, 61°17'24.95"W) and August 2014
115 (five nests from Verdant Vale and Eastern Main Road – 10°39'1.21"N, 61°15'9.63"W). Upon collection,
116 nests consisted of a single paper comb, with a mean (\pm standard error) number of 12.3 ± 2.0 adult
117 females and 111.3 ± 27.6 cells per comb (Supplementary Table S1). Nest membership was recorded
118 at least once per day for 5–22 days by marking individual wasps with honeybee queen tags and Uni
119 POSCA pens. Prior to nest collection, two female behavioural phenotypes were identified: a 'queen', by
120 removal of an egg and observing subsequent oviposition within the day; and a nonreproductive helper
121 'forager', by observing an individual bringing a solid mass of food back to the nest (in 2013) or by
122 recording which individuals spent the least amount of time on the nest (in 2014). In one nest, a queen
123 could not be identified following egg removal, and so instead the five females who spent the most time
124 on the nest during observations were selected as 'potential queens'. Nest collection occurred at dusk,
125 with adults and brood stored in 80% ethanol at -20°C.

126

127 The identified queens, a single forager, and a random sample of five pupae were genotyped per nest
128 (24 adults and 50 pupae total). DNA was extracted using a HotSHOT procedure (Truett et al., 2000;
129 Montero-Pau et al., 2008). We used 120 SNP-based markers (KASP™ LGC Genomics) developed for
130 *P. canadensis* and *P. lanio* (see Southon et al., 2019 for SNP-loci discovery and PCR protocol). First,
131 a subset of 35 pupae (five pupae each from seven nests) were genotyped at all 120 loci. From this
132 subset, monomorphic loci, loci with unclear clustering on a XY plot (MxPro™ Mx3005p® v4.10) and loci
133 with minor allele frequencies < 5% were removed from further analysis (removing 27 loci). All individuals
134 were genotyped, and genetic structure analysed at 93 loci (35 individuals) and 70 loci (39 individuals).
135 Using R 3.3.3 (R Core Team, 2013) packages 'adegenet' (Jombart, 2008; Jombart & Ahmed, 2011),
136 'genepop' (Rousset, 2008) and 'pegas' (Paradis, 2010), 10 genotypes (one individual per nest) were
137 assessed at 93 valid loci for deviations from Hardy–Weinberg equilibrium (Bonferroni corrected $\alpha =$

138 0.000538; 10,000 Monte Carlo permutations) and for linkage disequilibrium (log likelihood-ratio statistic,
139 $\alpha = 0.05$; 1,000 dememorizations; 100 batches; 1,000 iterations per batch).

140

141 Relatedness was assessed using the Wang (2002) and Lynch & Ritland (1999) metrics in
142 COANCESTRY 1.0.1.8 (Wang, 2011), excluding individuals identified as males (pupae with no
143 heterozygous loci). We report relatedness estimates using the Wang estimate; the Lynch & Ritland
144 estimate was used to produce individual inbreeding coefficients (F). A one-sample t-test ($\alpha = 0.05$) was
145 used to determine whether F values deviated from random mating ($F = 0$). SNP-based pedigrees were
146 constructed using COLONY 2.0.6.5 (Jones & Wang, 2010) on individuals that had first been sorted into
147 their two respective populations of Verdant Vale and Eastern Main Road (settings: female polygamy,
148 male monogamy, full-likelihood, very long run length, no updated allele frequency, 1% error rate).
149 Though female polygamy is rare in *Polistes*, it was included as low levels of multiple mating have been
150 previously detected in the genus (Strassmann, 2001; Seppä et al., 2011; Southon et al., 2019). In the
151 first model, queens and potential queens were designated as possible mothers, with foragers and pupae
152 as offspring. Queens and potential queens with no identified offspring were then designated as offspring
153 in a second model, and these two models combined to construct the complete pedigree. COLONY
154 assignment was accepted if cluster probability p was ≥ 0.8 , or if there was ≥ 0.5 probability between the
155 primary and the secondary substructures. To measure reproductive skew between the number of adult
156 females observed on the nest, expressed in genotyped pupae, we calculated the B index which ranges
157 from -1 (equally shared) to 1 (complete skew) (Nonacs, 2000) (SKEW CALCULATOR 2003 © Peter
158 Nonacs; settings: 1,000 simulations, 95% CI, $\alpha = 0.05$, equal length of time on nest assumed).

159

160 **Results**

161 For 93 valid loci, $96.9 \pm 0.2\%$ of alleles could be scored successfully across all individuals (see
162 Supplementary Table S2 & S3). All loci were in Hardy–Weinberg equilibrium (Bonferroni corrected α),
163 with linkage disequilibrium observed across 2.1% of 4,278 pairings ($n = 91$). Mean observed
164 heterozygosity was 0.44 ± 0.02 in Verdant Vale ($n = 8$, $H_{expected} = 0.42 \pm 0.01$) and 0.52 ± 0.04 in Eastern
165 Main Road ($n = 2$, $H_{expected} = 0.33 \pm 0.02$). Mean inbreeding coefficient of all females ($F = -0.03 \pm 0.02$,
166 range -0.27–0.34) was not significantly different from zero ($t_{70} = -1.98$, $p = 0.051$), suggesting random
167 mating.

168 Analyses indicated high levels of reproductive skew, and that presumed low conflict may be attributed
169 to the high nestmate relatedness among adults and/or chance of nest inheritance by daughters (who
170 could alternatively start new nests). A significant female reproductive monopoly was detected across
171 all nests ($B = 0.7191 \pm 0.0124$, $p < 0.05$, range 0.6667–0.7636). Mean relatedness r between female
172 nestmate pupae (overall means of 10 nests, see Supplementary Table S4) was 0.71 ± 0.01 (range
173 0.64–0.78). Overall mean COLONY assignment probability to pedigree structures was $p = 0.89 \pm 0.05$,
174 range 0.60–1.00. SNP-based pedigree reconstructions revealed six of the nests as having the identified
175 queen as the mother of the forager and pupae (assignment $p = 0.87 \pm 0.08$, range 0.60–1.00; two nests
176 $p < 0.8$ but substructure difference ≥ 0.5 ; Figure 1 – Panel 2). On three nests, a daughter appears to
177 have replaced (or be attempting to replace) the queen as the identified egg layer but was not the mother
178 to the pupae (assignment $p = 0.97 \pm 0.01$, range 0.95–0.98; Figure 1 – Panel 3). In four nests, an adult
179 offspring had the same mother but a different father to sibling pupae, suggesting some multiple mating
180 (Figure 1 – Panel 2B; Panel 3E/F). One nest had an undetermined pedigree structure, where the queen
181 was assigned as the mother of female pupae and an undetected not genotyped mother as the parent
182 of the forager and a male pupa (assignment $p = 0.78$, substructure difference > 0.5).

183

184 **Discussion**

185 Although *Polistes* paper wasps have a cosmopolitan distribution, genetic-structure data are critically
186 lacking for tropical species of the genus. This is especially so for data which allow a reconstruction of
187 nesting group genetic structure; for example, most studies on tropical species are based on allozyme
188 data (see Southon et al., 2019). In the Neotropical *P. lanio*, we found that female reproductive skew
189 and between-offspring relatedness in post-emergence nests are on average high. The presence of
190 replacement queens also indicates that natal nests are often inherited by daughters. Both high
191 relatedness and heritable skew in post-emergence nests fit trends found in temperate and tropical
192 *Polistes*.

193

194 High reproductive skew is typical in established post-emergence nests of temperate *Polistes* species,
195 suggesting that conflicts over reproduction at this stage in the nesting cycle are relatively resolved. As
196 nonreproducing female helpers (in this study, genotyped foragers) are often offspring of the resident
197 queen and related to the brood, conflict over who reproduces may be reduced as helpers gain indirect

198 fitness from raising siblings (Field et al., 1998; Reeve et al., 2000; Seppä et al., 2002). However, in
199 large nests (R.J.S. pers. obs. of *P. lanio* nests with 60+ adult members present, Trinidad 2015), indirect
200 fitness benefits may become reduced if helper to brood productivity is nonlinear (Nonacs, 1991;
201 Grinsted & Field, 2018). In our sampled nests, the number of adult females increased linearly with brood
202 number, but bigger nests do have a number of empty cells (see Supplementary Table S1). Brood
203 number may be limited by the queen (fecundity or a monopolisation strategy) or helper productivity. A
204 single genotyped forager was not directly related to the nest pupae may have been a remaining
205 foundress or drifter — a non-natal individual which visits multiple nests (Nonacs, 2017). These
206 individuals usually behave as helpers in tropical *Polistes* (Sumner et al., 2007). Whilst nest pupae
207 appear to be mostly related, there may be a potential number of unrelated adults on a nest.

208

209 Replacement queens (aka queen turnover) were also detected, often reported in genetic studies as
210 older and younger offspring belonging to different immediate matriline (Strassmann & Meyer, 1983;
211 Hughes et al., 1987; Peters et al., 1995). Given that we detected a high degree of queen replacement
212 among a relatively small sample size, it is likely that queen replacement is a common phenomenon in
213 this species. In complex societies with fixed castes and monopolised reproduction, queen loss may
214 signal the termination of the nesting cycle (Duchateau & Velthuis, 1989; Pirk et al., 2004). In simple
215 societies with flexible helpers, as in *Polistes*, replacement queens may inherit the nest and continue the
216 nesting cycle if there are no seasonal constraints. An alternative explanation for the presence of multiple
217 mothers is that there could occasionally be multiple queens (West-Eberhard, 1986).

218

219 Multiple mating by the queen was detected in four matriline, where in each case, two fathers were
220 detected within a single matriline, specifically the adult 'generation' and pupae belonged to separate
221 patriline. Low levels of multiple mating have sometimes been reported in *Polistes*, including in the
222 sister species *P. canadensis* from analyses with both SNP and microsatellite markers (Southon et al.,
223 2019); however, we cannot rule out the possibility that this could be a miscalculation attributed to how
224 COLONY models novel SNP genotypes. Occurrences of multiple mating and potential re-mating (due
225 to the temporal separation of adult and pupae offspring) warrant further investigation, for example, by
226 genotyping larger numbers of brood across multiple generations through the entire colony cycle.
227 Instances of multiple mating in a species without distinct morphological castes may provide support to

228 the supposition that *Polistes* societies should be viewed more as cooperative breeders than eusocial
229 entities (Boomsma, 2009; Boomsma & Gawne, 2018), but under what circumstances it arises and
230 ultimately if it has any impact on group social behaviour is unknown.

231

232 This study together with the parallel study on its sister species *P. canadensis* (Southon et al., 2019),
233 provides comprehensive genetic evidence that tropical species of *Polistes*, despite differences in
234 ecological conditions, experience similar resolution of reproductive conflict as temperate species. The
235 use of SNP genotyping reveals a range of pedigrees in post-emergence nests although at any one time
236 there is usually a single, singly-mated mother and her daughters, queen replacement (by daughters) is
237 common and there may be low levels of multiple mating. Such a range of pedigree structures detected
238 within just ten nests (limited to the beginning of the wet season) suggests that relationships in these
239 societies are often complex, and that broader sampling, within and between different times of the
240 season would be important to understand these better. Though general trends in reproductive skew
241 across the genus appear conserved, and it may be presumed that high reproductive skew is an
242 ancestral trait of *Polistes* from its Indomalayan origin (Santos et al., 2015).

243

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257

258 **Conflicts of interest/Competing interests**

259 None known.

260

261 **Availability of data and material**

262 See provided supplementary files.

263

264 **Code availability**

265 NA.

266

267 **Authors' contributions**

268 All authors contributed to the designing of the study and writing the manuscript. R.J.S. conducted field

269 studies and lab/data analyses.

270

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434 **Supplementary Files**

435 Further data on SNP loci discovery can be viewed at:
436 https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ss.cgi?subsnp_id=3023075125 .

437

438 Supplementary Table S1. Characteristics for post-emergence *P. lanio* nests used in this study.

439

440 Supplementary Table S2. Position, count, coverage and frequency for the 120 SNP loci discovered in
441 *P. canadensis* and *P. lanio* (Southon et al., 2019).

442

443 Supplementary Table S3. SNP genotypes of sampled individuals (valid 93 loci).

444

445 Supplementary Table S4. Pairwise Wang relatedness estimates per nest between pupae.

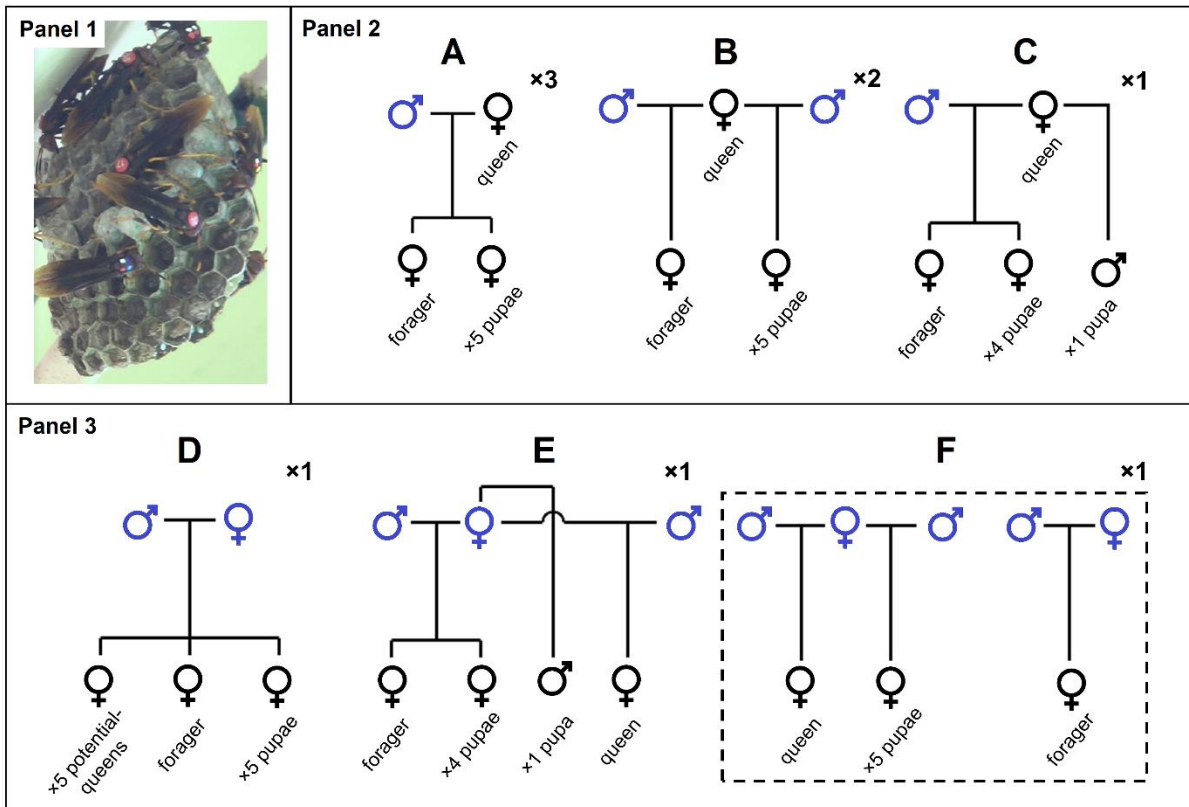
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448 **Figure Legend**

449 Figure 1. SNP pedigrees of nine *P. lanio* nests, each pedigree including genotypes (labelled) of an
450 identified queen or potential queens, forager and five pupae (number of nests per pedigree is given in
451 the upper right corner). **Panel 1.** Post-emergence *P. lanio* nest, in which many female helpers are
452 thought to be capable of becoming queens. **Panel 2.** Six nest pedigrees in this study had a single queen
453 who monopolised reproduction, with three variates: (A) a singly-mated queen; (B) a twice mated queen,
454 where the sampled forager had a different father but same mother to the pupae; (C) a singly-mated
455 queen, who was the mother to all sampled female and male offspring. **Panel 3.** Three nests showed
456 pedigrees in which likely queen replacement was observed, with three variates: (D) a singly-mated
457 “queen”, not genotyped/detected prior to nest collection, being the mother of all offspring including
458 individuals that showed potential queen-like behaviour (pre- egg laying, sitting around on nests); (E) the
459 mother of female and male offspring was not the queen detected from behaviour observations, which
460 was a half-sister to the other female offspring; (F) two separate matriline were detected, one was
461 mother to the sampled forager, the other a twice mated mother to the sampled queen and pupae – with
462 a generational split in paternity.

463



465

466 **Figure 1.**