

1 **Asymmetric assortative mating and queen polyandry are linked to a supergene**
2 **controlling ant social organization**

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11 **Abstract**

12 Non-recombining genomic variants underlie spectacular social polymorphisms, from bird
13 mating systems to ant social organization. Because these “social supergenes” affect multiple
14 phenotypic traits linked to survival and reproduction, explaining their persistence remains a
15 substantial challenge. Here, we investigate how large non-recombining genomic variants relate
16 to colony social organization, mating system and dispersal in the Alpine silver ant, *Formica*
17 *selysi*. The species has colonies headed by a single queen (monogynous) and colonies headed
18 by multiple queens (polygynous). We confirmed that a supergene with alternate haplotypes –
19 Sm and Sp – underlies this polymorphism in social structure: females from mature monogynous
20 colonies had the Sm/Sm genotype, while those from polygynous colonies were Sm/Sp and
21 Sp/Sp. Queens heading monogynous colonies were exclusively mated with Sm males. In
22 contrast, queens heading polygynous colonies were mated with Sp males and Sm males. Sm
23 males, which are only produced by monogynous colonies, accounted for 22.9% of the matings
24 with queens from mature polygynous colonies. This asymmetry between social forms in the
25 degree of assortative mating generates unidirectional male-mediated gene flow from the
26 monogynous to the polygynous social form. Biased gene flow was confirmed by a significantly
27 higher number of private alleles in the polygynous social form. Moreover, heterozygous queens
28 were three times as likely as homozygous queens to be multiply mated. This study reveals that
29 the supergene variants jointly affect social organization and multiple components of the mating
30 system that alter the transmission of the variants and thus influence the dynamics of the system.

31 **Introduction**

32 Supergenes are clusters of tightly linked loci controlling complex phenotypes (Dobzhansky,
33 1970; Schwander, Libbrecht, & Keller, 2014; Thompson & Jiggins, 2014). They underlie some
34 of the most spectacular polymorphisms in nature, including sexes (Charlesworth, 2016),
35 mimetic forms in butterflies (Joron et al., 2011), mating tactics in birds (Küpper et al., 2016;
36 Tuttle et al., 2016) and social organization in ants (Wang et al., 2013; Purcell, Brelsford, Wurm,
37 Perrin, & Chapuisat, 2014). Large non-recombining supergene variants are typically associated
38 with differences in survival and coordinated changes in multiple morphological, physiological
39 and behavioral traits (Schwander et al., 2014; Tuttle et al., 2016; Chouteau, Llaurens, Piron-
40 Prunier, & Joron, 2017). Because supergenes typically influence their own transmission in
41 complex ways, understanding which mechanisms contribute to the maintenance of
42 polymorphism is challenging (Llaurens, Whibley, & Joron, 2017). Fundamental mechanisms
43 stabilizing genetic polymorphisms include disassortative mating, heterozygote advantage and
44 spatially variable selection coupled with gene flow.

45 The recent discovery that convergent supergenes influence colony social organization in two
46 ant species provides a novel opportunity to investigate how supergenes contribute to
47 intraspecific phenotypic diversity (Wang et al., 2013; Libbrecht & Kronauer, 2014; Purcell et
48 al., 2014). These large non-recombining variants control whether one or multiple queens
49 reproduce within each colony. Variation in queen number shapes the degree of within-group
50 relatedness and thus influences the inclusive fitness of helpers (Hamilton, 1964; Crozier &
51 Pamilo, 1996; Ross, 2001; Bourke, 2011). Given the central importance of kinship for social
52 evolution, what causes variation in the number of queens reproducing in each colony has been
53 a long-standing puzzle for evolutionary biologists (Hölldobler & Wilson, 1977; Bourke &
54 Franks, 1995; Keller, 1995; Ross, 2001).

55 One solution to this puzzle is that the transition from one to multiple breeders per group may
56 be coupled with a reduction in dispersal, thereby reducing the erosion of within-group
57 relatedness (Nonacs, 1988; Bourke & Franks, 1995; Ross, 2001). A recent model shows that
58 social polymorphism readily emerges through linkage of loci involved in social behavior and
59 dispersal, pointing to the possibility that supergenes control both traits (Mullon, Keller, &
60 Lehmann, 2018). So far, the coupling of social organization and dispersal within a supergene
61 has not been directly investigated in an empirical system.

62 Across ant species, variation in social organization frequently correlates with shifts in dispersal
63 and mating strategies (Bourke & Franks, 1995; Ross, 2001). In species forming polygynous (=
64 multiple-queen) colonies, queens often mate nearby or within their natal nest and disperse on
65 foot and with nestmate workers to establish new nests in the vicinity (Keller, 1995; Crozier &
66 Pamilo, 1996; Chapuisat, Goudet, & Keller, 1997). In contrast, in species forming monogynous
67 (= single-queen) colonies, queens usually disperse on the wing, mate in swarms away from their
68 natal nest, establish an incipient colony and produce their first workers independently
69 (Timmermans, Grumiau, Hefetz, & Aron, 2010; Jowers et al., 2013). In line with a shift in
70 dispersal strategies, polygynous populations generally show higher levels of genetic
71 differentiation among populations, compared to monogynous populations (reviewed in Pamilo,
72 Gertsch, Thorén, & Seppä, 1997; Sundström, Seppä, & Pamilo, 2005). Whether parallel
73 differences in dispersal and mating strategies occur between monogynous and polygynous
74 colonies belonging to the same population deserves further investigation. A polymorphic

75 population also provides an opportunity to investigate whether social organization and dispersal
 76 covary with alternative variants at a supergene.

77 In the Alpine silver ant, *Formica selysi*, social organization is associated with a polymorphic
 78 supergene that is 14.1 Mbp long and contains 664 coding genes (Purcell et al., 2014; Avril,
 79 Tran, Brelsford, & Chapuisat, unpublished results). The supergene has two large non-
 80 recombining haplotypes, Sm and Sp, which are highly differentiated both in nucleotide
 81 sequence and gene order. The haplotypes differ by multiple inversions (Brelsford, Purcell, &
 82 Chapuisat, unpublished results), but it is not yet clear whether inversions are a cause or
 83 consequence of the arrest of recombination (Sun, Svedberg, Hiltunen, Corcoran, &
 84 Johannesson, 2017). Like in other Hymenoptera the females are diploid and the males haploid.
 85 Monogynous colonies produce diploid workers and gynes (winged females destined to become
 86 queens) that all have the Sm/Sm supergene genotype, along with haploid winged males carrying
 87 the Sm haplotype (Figure 1; Purcell et al., 2014; Purcell et al., 2016). In contrast, polygynous
 88 colonies produce Sm/Sp and Sp/Sp workers and gynes, along with winged males carrying
 89 exclusively Sp (Figure 1; Purcell et al., 2014; Purcell et al., 2016). The absence of Sm winged
 90 males produced by polygynous colonies suggests that the Sp haplotype is a selfish genetic
 91 element favoring its own transmission over the one of the alternative haplotype. Eggs from
 92 heterozygous queens that did not inherit Sp failed to hatch, indicating that Sp is a maternal-
 93 effect killer (Avril, Purcell, Béniguel, & Chapuisat, unpublished results). The unusual
 94 genotypic distribution of the supergene across social forms raises multiple questions. Do queens
 95 only mate with males originating from the same social form or is there some degree of non-
 96 assortative mating? What is the pattern of gene flow between social forms? What prevents the
 97 driving haplotype Sp from going to fixation?

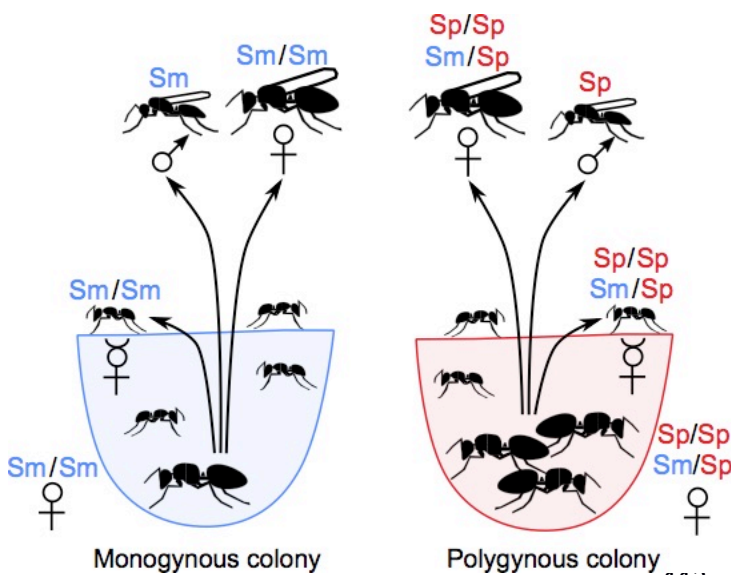


Figure 1. Genetic system underlying variation in social organization in the Alpine silver ant, *F. selysi*. Queens and workers are diploid females, males are haploid. Sm and Sp indicate alternative non-recombining variants (i.e., haplotypes) at a supergene associated with colony social structure. In short, workers and queens in mature polygynous colonies have one or two copies of the Sp haplotype, while workers and queens in mature monogynous colonies lack Sp. Indeed, females established in mature monogynous colonies have the supergene genotype Sm/Sm.

114 Monogynous colonies produce Sm/Sm workers and gynes (winged females destined to become
 115 queens), along with Sm males. Females established in mature polygynous colonies have the
 116 supergene genotypes Sm/Sp and Sp/Sp. Polygynous colonies (= groups headed by Sm/Sp and
 117 Sp/Sp queens) produce Sm/Sp and Sp/Sp workers and gynes, along with Sp males. The absence
 118 of Sm males and Sm/Sm females in brood produced by polygynous colonies indicates that Sp is
 119 a transmission ratio distorter and raises the question, do Sm/Sp queens mate with Sm males?

120 Field observations indicate that queens and males of both social forms fly away from their natal
121 colony and mate on top of small trees located nearby (Chapuisat, Bocherens, & Rosset, 2004;
122 Rosset & Chapuisat, 2006, 2007). Whether queens and males from polygynous colonies also
123 mate within their natal colony as an alternative strategy is presently unknown. The genetic
124 structure at microsatellite markers did not reveal marked differences between social forms in
125 mating or dispersal (Chapuisat et al., 2004; Purcell & Chapuisat, 2013). Moreover, the absence
126 of differentiation between the monogynous and polygynous social forms at both microsatellites
127 and SNPs located outside of the supergene suggests that the social forms are not reproductively
128 isolated (Chapuisat et al., 2004; Purcell & Chapuisat, 2013; Purcell et al., 2014).

129 Here, we investigate how the supergene variants relate to colony social organization, mating
130 system and dispersal in the Alpine silver ant. We use genomic data from offspring of isolated
131 queens to examine whether differences in mating and dispersal of queens and males from each
132 social form affect the transmission of alternative variants of the supergene. We pursue three
133 main goals. First, we quantify the degree of assortative mating with respect to social origin. In
134 particular, we examine whether queens heading mature monogynous and polygynous colonies,
135 respectively, mated with males from the same or the alternative social origin. Second, we
136 investigate whether queens and males of each social origin differ in their propensity to mate
137 locally, causing differences in patterns of isolation by distance. Finally, we compare the
138 frequency of private alleles and use Bayesian inference to assess gene flow asymmetries
139 between social forms. These results will reveal if some degree of non-assortative mating or
140 unusual patterns of gene flow affect the dynamics of this “social” supergene.

141 **Materials and methods**

142 *Sampling and genotyping strategy*

143 The Alpine silver ant *Formica selysi* is a socially polymorphic species that inhabits large valleys
144 in the Alps and the Pyrenees (Chapuisat et al., 2004; Purcell, Pellissier, & Chapuisat, 2015).
145 The study population is located in central Valais, Switzerland (Finges; 7°36'30" E, 4°18'30"
146 N, altitude: 565 m; Figure S1). The social organization (monogynous or polygynous) of
147 colonies included in the present study had been previously determined by genotyping nestmate
148 workers at microsatellite markers (Purcell & Chapuisat, 2013). It was further confirmed by
149 counting queens during sampling and analyzing single nucleotide polymorphisms (SNPs)
150 located outside and within the supergene (see below).

151 To infer the genotypes of queens and their mates, we genotyped offspring of single queens (=
152 progenies) and reconstructed the parental genotypes. This strategy was chosen to circumvent
153 the difficulty and destructive nature of sampling mature queens from monogynous colonies.
154 Therefore, workers from monogynous colonies were sampled from the field. A similar
155 genotyping of progeny was applied to queens from polygynous colonies in order to obtain
156 comparable data on mating frequency and mate genotypes for both social forms. We thus
157 analyzed workers and eggs from queens sampled in polygynous field colonies and reared singly
158 in laboratory colonies (Figure S2).

159 The sampling and genotyping strategy combined RAD-seq genotyping of worker progenies
160 from single (isolated) queens and PCR-RFLP assay of queens, mates and eggs (Figure S2; Table
161 S1). Progenies from monogynous queens consisted of four workers sampled from each of 63
162 monogynous colonies (Figure S1; Table S1). Progenies from polygynous queens were obtained

163 by isolating 142 wingless reproductive queens sampled from 51 polygynous colonies (Figure
164 S1; Table S1). To minimize the impact of sampling, we left at least two observed queens in
165 each polygynous colony. Each sampled queen was placed individually in a small plastic box
166 (15 × 13 × 6 cm), with 20 adult workers from the same parent colony. The ants were provided
167 with a nest site, water and *ad libitum* ant food (Meunier & Chapuisat, 2009). Brood production
168 was monitored daily. Four callow (young) workers per queen were collected for 120 queens
169 originating from 37 polygynous colonies.

170 We obtained RAD-seq data for four workers per queen coming from 63 monogynous and 37
171 polygynous colonies, respectively (Table S1; Figure S2). The RAD-seq data were used to
172 reconstruct the genotypes of the live queens and their mates at SNPs outside of the supergene
173 and in the supergene (Table S1). The SNPs outside of the supergene were used to determine
174 queen mating frequency and for all population genetic analyses. The supergene genotype was
175 used to determine the social origin of the queens and their male mates (Table S1).

176 In addition to the four callow workers, we collected at least eight eggs per queen for all queens
177 from polygynous colonies (Figure S2). At the end of the experiment, we dissected the queens
178 and extracted the sperm contained in their spermathecae (Chapuisat, 1998). The supergene
179 genotypes of queens, sperm and eggs from polygynous colonies were determined with a PCR-
180 RFLP assay that discriminates three SNPs diagnostic for alternative haplotypes of the
181 supergene (Purcell et al., 2014). These RFLP data were used to confirm the supergene
182 genotypes of queens and mates inferred from RAD-seq data and to supplement the mating
183 pattern data (Table S1).

184 DNA was extracted from the head of queens and from the head and thorax of workers with
185 Qiagen Blood and Tissue extraction kit (Qiagen, Hombrechtikon, Switzerland). DNA from
186 eggs and sperm was extracted with a salting-out procedure (Miller, Dykes, & Polesky, 1988).

187 *Genotyping-by-sequencing*

188 We used a genotyping-by-sequencing (RAD-seq) approach to identify SNPs in workers
189 (Brelsford, Dufresnes, & Perrin, 2016; Purcell et al., 2016). The DNA was digested with
190 restriction enzymes MseI and SbfI. This combination of enzymes produced a low density of
191 SNP markers, which allowed us to multiplex the 732 workers on a single lane of Illumina HiSeq
192 2500 with an average coverage of 197 reads per locus per individual. The sequencing was
193 performed at the Lausanne Genomic Technology Facility in Lausanne, Switzerland.

194 The genetic data were processed with the software pipeline Stacks v1.46 (Catchen, Hohenlohe,
195 Bassham, Amores, & Cresko, 2013). The raw reads were demultiplexed using the
196 `process_radtags` module, and 22 individuals that had low numbers of reads (< 10,000) were
197 removed from the dataset. Reads were aligned to a reference genome with BWA v0.7.13 (H. Li
198 & Durbin, 2009). SNPs and genotypes were called with the `ref_map` module of Stacks. To avoid
199 bias due to linkage disequilibrium between adjacent markers, one SNP per RAD tag was
200 randomly selected, using VCFtools v0.1.14 (Danecek et al., 2011). The SNPs in the supergene,
201 which are linked, were retained but were analyzed separately from the ones outside of the
202 supergene. Genotypes with a quality score below 20 were treated as missing data. SNPs with a
203 minor allele frequency below 0.01 or missing for more than 20% of the individuals were
204 removed from the dataset. The final dataset included 271 SNPs, of which 25 were in the
205 supergene and 246 in the rest of the genome.

206 *Parental genotype reconstruction*

207 For each sibship (progenies from singly mated queens), the genotypes of the queen and her
208 male mate were reconstructed from RAD-seq data using the computer program COLONY
209 v2.0.6.1 (Jones & Wang, 2010). For population genomic analyses we conserved the 246
210 generated SNPs located outside of the supergene and excluded the 25 SNPs in the supergene.
211 Parental genotypes at given SNPs for which the posterior probability was below 0.8 were
212 considered missing values. For multiply mated queens, the maternal and paternal genotypes
213 could not be unambiguously reconstructed. These queens and their male mates were excluded
214 from population genetic analyses on sex-specific dispersal (section 2 below). In total, we
215 reconstructed the genotypes at SNPs outside of the supergene for 157 singly mated queens and
216 their mates.

217 *Genetic data analyses*

218 1. Social structure, supergene genotypes, mating pattern and queen mating frequency

219 The social structure of each colony had been previously inferred from microsatellite genotypes
220 of worker nestmates (Chapuisat et al., 2004; Purcell & Chapuisat, 2013). It was confirmed by
221 direct observation of queens in the field (polygynous colonies) and by measuring the relatedness
222 among nestmates (monogynous colonies). The supergene genotype of each queen and
223 respective male mate(s) was inferred from the supergene genotype of the worker progeny (25
224 SNPs in the supergene obtained by RAD-seq; Table S1). For queens and mates from
225 polygynous colonies, the supergene genotypes were further assessed with a PCR-RFLP assay
226 of queens, sperm and eggs (Purcell et al., 2014; Table S1).

227 We calculated the maximum likelihood relatedness among workers from single queens
228 (progenies) with the algorithm of Huang et al. (2015), implemented in the software
229 PolyRelatedness v1.6. To obtain unbiased estimate of relatedness, we used RAD-seq generated
230 SNPs located outside of the supergene. We estimated background allele frequencies with
231 colonies being weighted equally. Using these frequencies, we simulated 1,000 datasets
232 consisting of full-sibs (i.e. offspring from single-mated queens), calculated their relatedness and
233 estimated the 95% confidence interval around 0.75, the expected relatedness for full-sibs in
234 haplo-diploids. In complement to the relatedness analysis, we inferred the pedigree
235 relationships between sampled workers using the maximum likelihood approach implemented
236 in the program COLONY v2.0.6.1 (Jones & Wang, 2010). This method identifies full-sib and
237 half-sib groups. Consensus pedigree relationships were obtained from five iterations, with a
238 genotyping error rate set up at 0.01 per locus.

239 Queens were inferred to be singly mated when their worker progeny (i) had a relatedness not
240 significantly different from 0.75; and (ii) belonged to a full-sib group in the pedigree
241 reconstruction. Conversely, queens were assessed to be multiply mated when their worker
242 progeny had a relatedness significantly lower than 0.75 and belonged to a half-sib group. One
243 doubly mated queen had progeny with relatedness estimate not significantly different from
244 0.75. Due to the small number of offspring genotyped, the number of mates per queen and the
245 proportion of multiply-mated queens are minimum estimates (Boomsma & Ratnieks, 1996).
246 With four offspring, there is a 0.125 probability of not sampling a patriline when a queen had
247 mated with two equally contributing males. However, because we genotyped the same number

248 of offspring per queen, we can still compare the relative mating frequencies of queens with
249 alternative social genotypes.

250 2. Dispersal of queens and males

251 To get insight into the mating pattern we estimated the relatedness of the male mate to the queen
252 with the computer program PolyRelatedness v1.6. To test whether the male mate to queen
253 relatedness differs between social forms, we used a linear mixed model with the mate to queen
254 relatedness as response variable, queen and male social origin as fixed factors and the colonies
255 from which queens were sampled as a random factor. The model was built with the ‘lme4’ R
256 package (Bates, Mächler, Bolker, & Walker, 2015).

257 We estimated the relatedness among nestmate queens, among male mates of nestmate queens
258 and among progenies of nestmate queens with the computer program PolyRelatedness v1.6. To
259 estimate background allele frequencies, colonies were weighted equally. We simulated 1,000
260 datasets of unrelated individuals and computed the 95% confidence intervals around the
261 relatedness of zero corresponding to this null hypothesis.

262 Dispersal of queens and males was inferred by computing isolation by distance. The kinship
263 coefficient between pairs of individuals was regressed against the natural logarithm of distance.
264 We used Loiselle kinship coefficient because it is not affected by the ploidy of individuals,
265 thereby allowing us to compare the magnitude of isolation by distance between sexes (Loiselle,
266 Sork, Nason, & Graham, 1995; Hardy, Pearcy, & Aron, 2008). Regression was restricted to a
267 maximal distance of 900 meters to ensure that the computation was performed on a similar scale
268 for all comparisons. Correlation between the genetic and geographic matrices was tested with
269 a Mantel test with 10,000 permutations.

270 3. Gene flow between social forms

271 The amount of genetic differentiation between social forms was estimated using hierarchical *F*-
272 statistics, with workers nested in sibships, sibships nested in colonies, and colonies nested in
273 social forms. Calculation was performed with the Hierfstat R package v0.04-22 (Goudet, 2005).
274 Confidence intervals were obtained from 10,000 bootstrap resamples of loci.

275 We estimated the number of private alleles in workers from monogynous and polygynous
276 colonies, respectively (Slatkin, 1985). To control for the effects of unequal samples sizes and
277 hierarchical sampling, we bootstrap resampled the same number of workers in the monogynous
278 and polygynous social form, using only one individual per colony (Kalinowski, 2004). We
279 computed the number of private alleles in each social form with the R package ‘poppr’, based
280 on 10,000 bootstrap resamples (Kamvar, Tabima, & Grunwald, 2014). We used a permutation
281 test to evaluate whether the number of private alleles differed significantly between social
282 forms.

283 To estimate the number of immigrants per generation between social forms, we used the
284 Bayesian approach implemented in the computer program MIGRATE v3.6.11 (Beerli &
285 Palczewski, 2010). MIGRATE uses coalescent theory to estimate population genetic
286 parameters under the assumption of mutation-migration-drift equilibrium. The number of
287 immigrants per generation is calculated as the product between the mutation-scaled effective
288 population size within a focal social form and the mutation-scaled migration rate from the focal

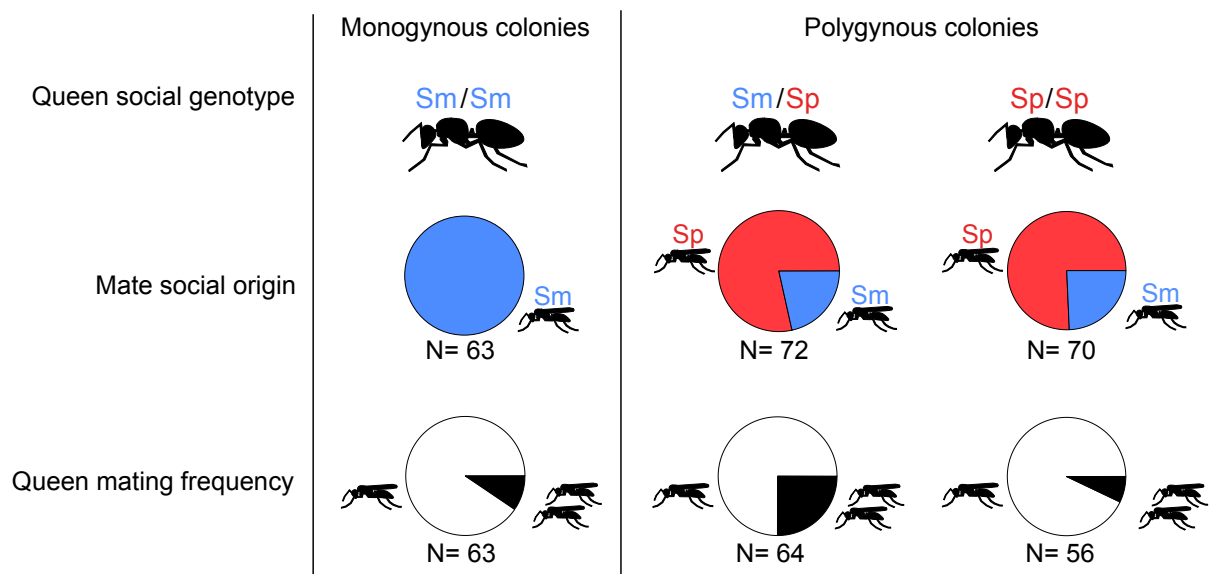
289 social form to the other social form. We ran MIGRATE with 20,000 burnin and 1,000,000
290 iterations.

291 Results

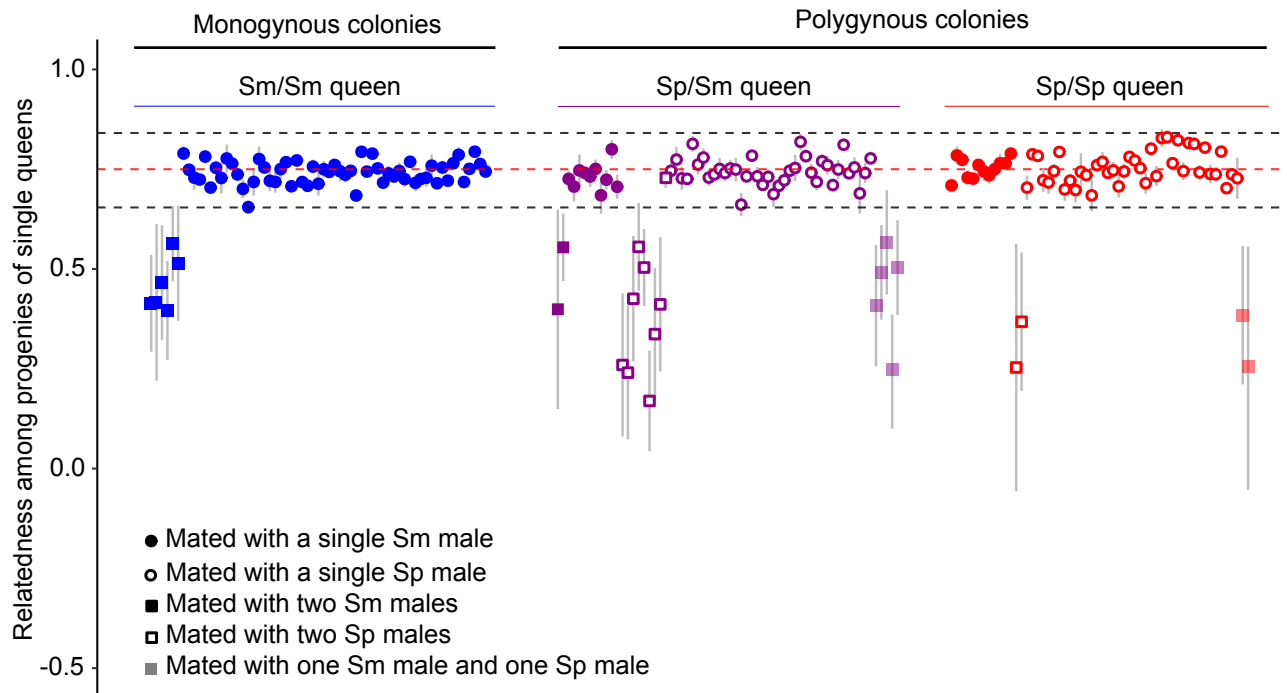
292 1. Social structure, supergene genotypes, mating pattern and queen mating frequency

293 The presence of a single reproducing queen in monogynous colonies was confirmed by the
294 relatedness among nestmate workers being close to 0.75 and by pedigrees consistent with a
295 single queen (Table S1). The presence of multiple queens in polygynous colonies was
296 demonstrated by direct sampling and observation of multiple wingless reproductive queens.
297 Social organization was perfectly associated with the genotypes of queens at the supergene: all
298 queens from monogynous colonies were Sm/Sm, and all queens from polygynous colonies had
299 at least one Sp haplotype, 51.3% being Sm/Sp and the rest Sp/Sp (Figure 2; Figure 3; Table
300 S1).

301 Queens heading mature monogynous colonies were invariably mated with Sm males (Figure 2;
302 Figure 3; Table S1). In contrast, queens in polygynous colonies were mated with Sm and Sp
303 males, with a relative contribution of Sm males totaling 22.9% (Figure 2; Figure 3; Table S1).
304 The proportion of mating with Sm versus Sp males did not differ significantly between Sp/Sp
305 and Sm/Sp queens (Figure 2; Fisher exact test, $p = 1$).



306 **Figure 2.** Social genotypes of queens and their male mates heading mature field colonies. Blue
307 and red pie charts indicate the proportion of mating with Sm males (blue) and Sp males (red),
308 respectively. White and black pie charts indicate the proportion of single mating (white) and
309 multiple mating (black) by queens. N is the number of queens. See Table S1 for details.

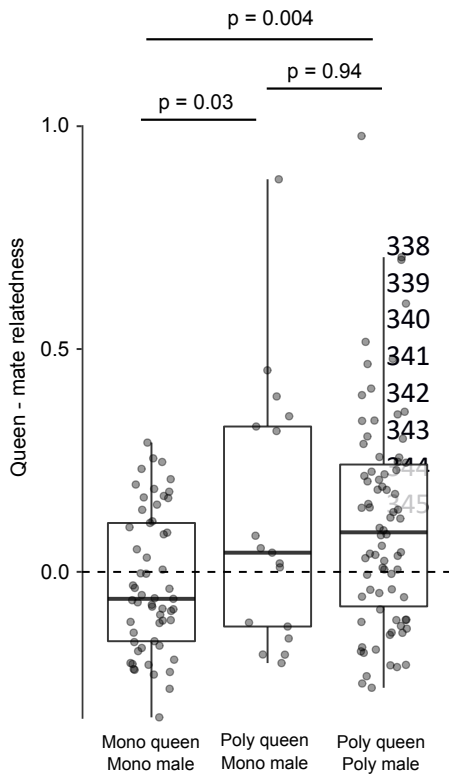


310 **Figure 3.** Social structure, supergene genotype and queen mating frequency. The y axis shows
 311 the relatedness (mean \pm SE) among progenies of single queens originating from monogynous
 312 colonies (left side of graph) and polygynous colonies (right side of graph), respectively. Red
 313 and black dotted lines indicate the mean and 95% confidence interval of the expected
 314 relatedness among progenies of a singly mated queen. Blue, purple and red symbols represent
 315 progenies of Sm/Sm queens, Sp/Sm queens, and Sp/Sp queens, respectively. Filled and open
 316 symbols represent progenies of queens mated with Sm and Sp males, respectively. Shaded
 317 symbols represent progenies of queens mated with one Sp and one Sm male. Circles stand for
 318 progenies of singly mated queen. Squares indicate progenies of multiply mated queens.

319 Most queens were singly mated (Figure 2; Figure 3; Table S1). Yet, at least 16.7% of the queens
 320 in polygynous colonies and 9.5% of the queens in monogynous colonies were mated with two
 321 males (Figure 2; Fisher exact test, $p = 0.26$). Given the small number of offspring genotyped,
 322 these observed mating frequencies are underestimates of actual mating frequencies. Notably,
 323 despite small sample size the observed mating frequency of queens in monogynous colonies is
 324 in line with previous estimates based on larger sample sizes in the same population (Chapuisat
 325 et al., 2004; Purcell & Chapuisat, 2013). The rate of multiple mating was associated with the
 326 social genotype of the queens. Heterozygous Sm/Sp queens were significantly more likely to
 327 be multiply mated than homozygous Sp/Sp and Sm/Sm queens (Figure 2; Figure 3; Fisher exact
 328 test, $p = 0.012$).

329 2. Dispersal of queens and males

330 The relatedness between queens and their male mates depended on the social origin of queens
 331 (Figure 4; Linear mixed model; $F_{(2,107)} = 5.80$, $p = 0.0041$). The relatedness of male mates to
 332 queens in polygynous colonies was significantly higher than the relatedness of male mates to
 333 queens in monogynous colonies (Figure 4). This pattern suggests that some queens from
 334 polygynous colonies mated with related Sp males originating from their own colony or a nearby
 335 polygynous colony, while other queens might have mated with related Sm males produced by
 336 the same monogynous colony in the neighborhood over multiple years. In contrast, queens from
 337 monogynous colonies mated with non-relatives.



338 **Figure 4.** Relatedness of male mates to queens: mates of
 339 monogynous origin to queens in monogynous colonies (left
 340 bar), mates of monogynous origin to queens in polygynous
 341 colonies (central bar) and mates of polygynous origin
 342 to queens in polygynous colonies (right bar). Boxplots
 343 represent the lower and upper quartiles and whiskers the
 344 minimum and maximum values (in the limits of $1.5 \times$
 345 interquartile range).

346 Two lines of evidence indicate that queens in polygynous colonies were related, as expected if
 347 some of these queens had stayed within or close to their natal colony. First, the relatedness
 348 among nestmate queens was significantly greater than zero ($r = 0.179 \pm 0.018$; mean \pm SE; $p <$
 349 0.05), while the relatedness among their mates was not ($r = 0.056 \pm 0.007$; mean \pm SE; $p >$
 350 0.05). Second the relatedness between progenies of queens belonging to the same polygynous
 351 colony was significantly greater than zero ($r = 0.117 \pm 0.089$; mean \pm SE; $p < 0.05$; Table S1).

352 For both social forms, patterns of isolation by distance revealed signs of restricted dispersal for
 353 queens, but not for males (Table 1). Indeed, the kinship coefficient decreased significantly with
 354 geographic distance for queens heading monogynous colonies and for queens heading
 355 polygynous colonies. In contrast, no significant isolation by distance was detected for males of
 356 monogynous origin, nor for males of polygynous origin (Table 1).

357

358 **Table 1.** Isolation by distance for queens and males belonging to each social form. R^2 is the
 359 correlation between kinship coefficient and geographic distance, b the slope of the regression
 360 and p the significance of the Mantel test.

	R^2	b	p
Monogynous social form			
Queens	-0.06	-0.0056	0.011
Males	-0.022	-0.000044	0.11
Polygynous social form			
Queens	-0.048	-0.0082	0.003
Males	0.022	0.0007	0.12

361 3. Gene flow between social forms

362 There was little genetic differentiation between social forms at SNPs located outside of the
 363 supergene ($F_{ST} = 0.0021$, 95% confidence interval [0.0003, 0.0039]). This absence of
 364 differentiation is in line with previous findings based on microsatellites and suggests ongoing
 365 gene flow between social forms (Chapuisat et al., 2004; Purcell & Chapuisat, 2013). The private
 366 allele analysis was consistent with unidirectional gene flow from the monogynous to the
 367 polygynous social form. Indeed, there were significantly more private alleles in workers of the
 368 polygynous social form than in workers of the monogynous social form (Figure 5). The
 369 Bayesian estimates of migration also suggest biased gene flow, with twice as many immigrants
 370 per generation from the monogynous to the polygynous social form compared to the reverse
 371 direction (N_m from the monogynous to the polygynous social form: median = 8.22, 95%
 372 confidence interval [4.39, 11.7]; N_m from the polygynous to the monogynous social form:
 373 median = 3.7, 95% confidence interval [1.82, 5.89]).

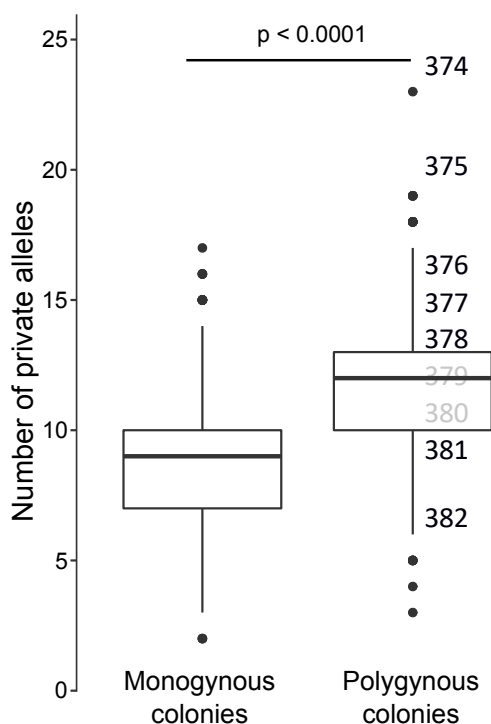


Figure 5. Number of private alleles in workers from monogynous and polygynous colonies, respectively, based on bootstrap resamples with one worker per colony. Boxplots represent the lower and upper quartiles and whiskers the minimum and maximum values (in the limits of $1.5 \times$ interquartile range).

383 **Discussion**

384 Genomic rearrangements associated with extended regions of suppressed recombination
385 underlie spectacular alternative phenotypes within populations (Schwander et al., 2014; Küpper
386 et al., 2016; Llaurens et al., 2017). Diverse mechanisms contribute to stabilize these
387 polymorphic supergenes, generally through some form of heterozygous advantage or negative
388 frequency-dependent selection (Llaurens et al., 2017). In many supergenes, the mutant
389 haplotype is a recessive lethal that confers some reproductive advantage to heterozygous
390 individuals (Wang et al., 2013; Schwander et al., 2014; Küpper et al., 2016). In other cases,
391 disassortative mating balances the polymorphism (Li et al., 2016; Tuttle et al., 2016; Chouteau
392 et al., 2017; Branco et al., 2018). In the Alpine silver ant, *Formica selysi*, a large genomic
393 polymorphism is associated with social organization (Purcell et al., 2014). Both homozygotes
394 are viable and the factors contributing to the maintenance of the polymorphism remain
395 mysterious.

396 To gain insights into the dynamics of alternate supergene haplotypes controlling social
397 organization, we investigated the mating system and dispersal strategies of queens and males
398 belonging to alternative social forms of the Alpine silver ant. We combined field sampling of
399 queens, RAD-seq genotyping of worker progenies and PCR-RFLP assays discriminating
400 alternative haplotypes of the supergene in queens, sperm and eggs. Together, these data
401 confirmed that colony social structure – being headed by one or by multiple reproductive queens
402 – was perfectly associated with alternative genotypes at a large supergene (Purcell et al., 2014).
403 The Sp haplotype was present in all queens heading multiple-queen colonies, with Sp/Sp and
404 Sm/Sp queens in similar proportions, while all queens heading single-queen colonies were
405 Sm/Sm. This unusual genotypic distribution in mature colonies prompted us to further
406 investigate the mating system, with a focus on whether mating in polygynous colonies is
407 assortative or disassortative with respect to the supergene haplotype.

408 The genotypes of queens and their mates revealed an asymmetry between social forms in the
409 frequency of non-assortative mating. Queens heading polygynous colonies were mated with Sp
410 males and Sm males, with the latter contributing to 22.9% of the mating (calculated weighting
411 all queens equally, independently on whether they had mated singly or multiply). This is an
412 intriguing mating pattern, because polygynous colonies do not produce Sm males (Figure 1 ;
413 Purcell et al., 2014 and unpublished data: 94 males from 21 polygynous colonies were all Sp).
414 We conclude that a fraction of polygynous queens mate with Sm males originating from
415 monogynous colonies. In contrast, all queens heading mature monogynous colonies were mated
416 with Sm males. We did not detect even a single case of non-assortative mating in monogynous
417 colonies, i.e. Sm/Sm queen mated with Sp male. The cause of this absence remains to be
418 investigated. Possible mechanisms include mate choice, mate availability, underdominance or
419 genetic incompatibilities. Alternatively, crosses between Sm/Sm queens and Sp males may be
420 transient, the incipient colony being converted into a polygynous colony headed by multiple
421 Sm/Sp daughter queens.

422 The asymmetric pattern of mating between social forms is expected to result in unidirectional
423 male-mediated gene flow from the monogynous to the polygynous social form. In line with this
424 prediction, we detected more private alleles in the polygynous social form than in the
425 monogynous social form. Moreover, the Bayesian estimate of the number of immigrants per
426 generation was twice as high from the monogynous to the polygynous social form than in the
427 other direction. Genetic differentiation between social forms was close to zero at markers

428 located outside of the supergene, indicating that gene flow is strong enough to homogenize
429 allelic frequencies between social forms, as already reported in previous studies of the same
430 population (Chapuisat et al., 2004; Purcell & Chapuisat, 2013; Purcell et al., 2014).

431 Complete assortative mating in the monogynous social form coupled with partial assortative
432 mating in the polygynous social form generates a mating advantage to Sm males, which runs
433 counter to the drive favoring the Sp haplotype (maternal-effect killing; Avril, Purcell, Béniguel,
434 & Chapuisat, unpublished results). Whether the combined selective forces maintain a
435 polymorphism, and the conditions under which they do so, requires modeling. Preliminary
436 results suggest that additional selective forces are needed to balance the polymorphism, as
437 mating biases against Sp when rare and transmission ratio distortion against Sm when rare
438 hinder polymorphism (Ghaseminejad, Chapuisat, & Otto, unpublished result). A similar pattern
439 of male-mediated unidirectional gene flow from monogynous to polygynous populations has
440 been documented in *Solenopsis invicta*, another ant species where social organization is
441 determined by a supergene (Ross & Keller, 1995; Shoemaker & Ross, 1996; Wang et al., 2013).
442 Interestingly, in *S. invicta* the haplotype causing polygyny favors its own transmission through
443 a green beard effect (Keller & Ross, 1998). One difference between the two systems is that in
444 *F. selysi* biased gene flow occurs among colonies belonging to the same local population.

445 *F. selysi* queens with alternative genotypes at the supergene differed in their rate of polyandry.
446 Specifically, the occurrence frequency of multiple mating was three times higher for Sm/Sp
447 queens than for homozygous queens. Mating with a single Sm male is detrimental for
448 heterozygous queens, because the Sp haplotype is a maternal-effect killer causing
449 developmental arrest of their Sm/Sm brood (Avril, Purcell, Béniguel, & Chapuisat, unpublished
450 results). Mating with multiple males might be a form of bet-hedging to mitigate the costs
451 induced by the driving haplotype, as suggested for the *t*-locus in mice (Sutter & Lindholm,
452 2015). More generally, polyandry occurs in response to the low fertility of males carrying the
453 driving haplotypes in multiple systems, including fire ants (Lawson, Vander Meer, &
454 Shoemaker, 2012; Wedell, 2013; Holman, Price, Wedell, & Kokko, 2015). Low fertility of Sp
455 males coupled with the cost of maternal-effect killing may contribute to the elevated rate of
456 polyandry in heterozygous queens. Whether heterozygous queens are more attractive than
457 homozygous queens and whether higher mating frequency provides a fitness advantage to
458 heterozygous queens remain to be investigated.

459 Three complementary methods to infer dispersal propensities of males and queens of
460 monogynous and polygynous origin provided limited support for the predicted link between
461 supergene variant, social structure and dispersal (Mullon et al., 2018). In contrast to the model
462 prediction, genetic data from queens and their mates did not reveal major differences in
463 dispersal between alternative social forms. Queens of both social forms showed signs of
464 isolation by distance, consistent with restricted dispersal. Isolation by distance was lower and
465 not significant for males, suggesting that males of both social forms are better dispersers than
466 queens. Such a difference between sexes has already been documented in ants – males can
467 disperse long distances on the wing and any post-mating dispersal by queens carrying sperm
468 stored in their spermathecae will further contribute to disperse male genes (Sundström, Keller,
469 & Chapuisat, 2003; Holzer, Keller, & Chapuisat, 2009). Yet, in line with the expectation that
470 queens of polygynous origin (bearing the Sp variant) should be more philopatric than queens
471 of monogynous origin (lacking the Sp variant), two lines of evidence suggest that queens from
472 polygynous colonies tend to mate nearby and often stay in their natal colony. First, the
473 relatedness between queens and their mates was higher for queens in polygynous colonies than

474 for queens in monogynous colonies. Second, nestmate queens in polygynous colonies were
475 significantly related. Local mating and establishment in natal colony appears to be common to
476 many polygynous ant species and is associated with large colony size, long colony lifespan and
477 colony propagation by budding, a process whereby queens and workers depart on foot to jointly
478 establish a new colony nearby (Nonacs, 1988; Bourke & Franks, 1995; Keller, 1995; Rosset &
479 Chapuisat, 2007). This alternative dispersal strategy likely contributes to the success and
480 persistence of the polygynous social organization.

481 **Conclusion**

482 Large non-recombining genomic variants underlying alternative social systems typically affect
483 multiple behavioral traits, including cooperative behavior, aggression and mate choice (e.g.
484 Tuttle et al., 2016). They can influence their own transmission in multiple ways and have
485 complex effects across multiple levels of biological organization. Here we showed that the
486 supergene controlling social organization in the Alpine silver ant is linked to major differences
487 in the mating system. First, heterozygous queens were three times as likely as homozygous
488 queens to be multiply mated. Second, males and females of alternative social forms differed in
489 their propensity to mate with the opposing social form. Specifically, males of monogynous
490 origin mated with both types of queens, while queens of polygynous origin mated with both
491 types of males. The asymmetry in the amount of assortative mating provides a mating advantage
492 to males of monogynous origin, which runs counter to the transmission ratio distortion that
493 favors the haplotype underlying polygynous social structure. These opposing forces will greatly
494 affect the dynamics of the genetic polymorphism controlling ant social organization.

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503 **Data accessibility**

504 Demultiplexed genotyping-by-sequencing reads have been deposited in the NCBI Sequence
505 Read Archive under the accession number SRP150340. Table S1 in supporting information
506 describes all samples, genetic analyses and social genotypes of queens heading monogynous or
507 polygynous colonies, their worker progeny, eggs and male mates, respectively.

508 **Author contribution**

509 A.A. J.P. and M.C. designed the study. A.A. performed the experiments and laboratory
510 analyses. A.A. analyzed the data with input from all authors. A.A. and M.C. wrote the
511 manuscript with input from J.P. and A.B.

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MOLECULAR ECOLOGY

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Figure S1. Map of monogynous (blue) and polygynous (red) colonies from which four workers per queen were collected for RAD-sequencing.

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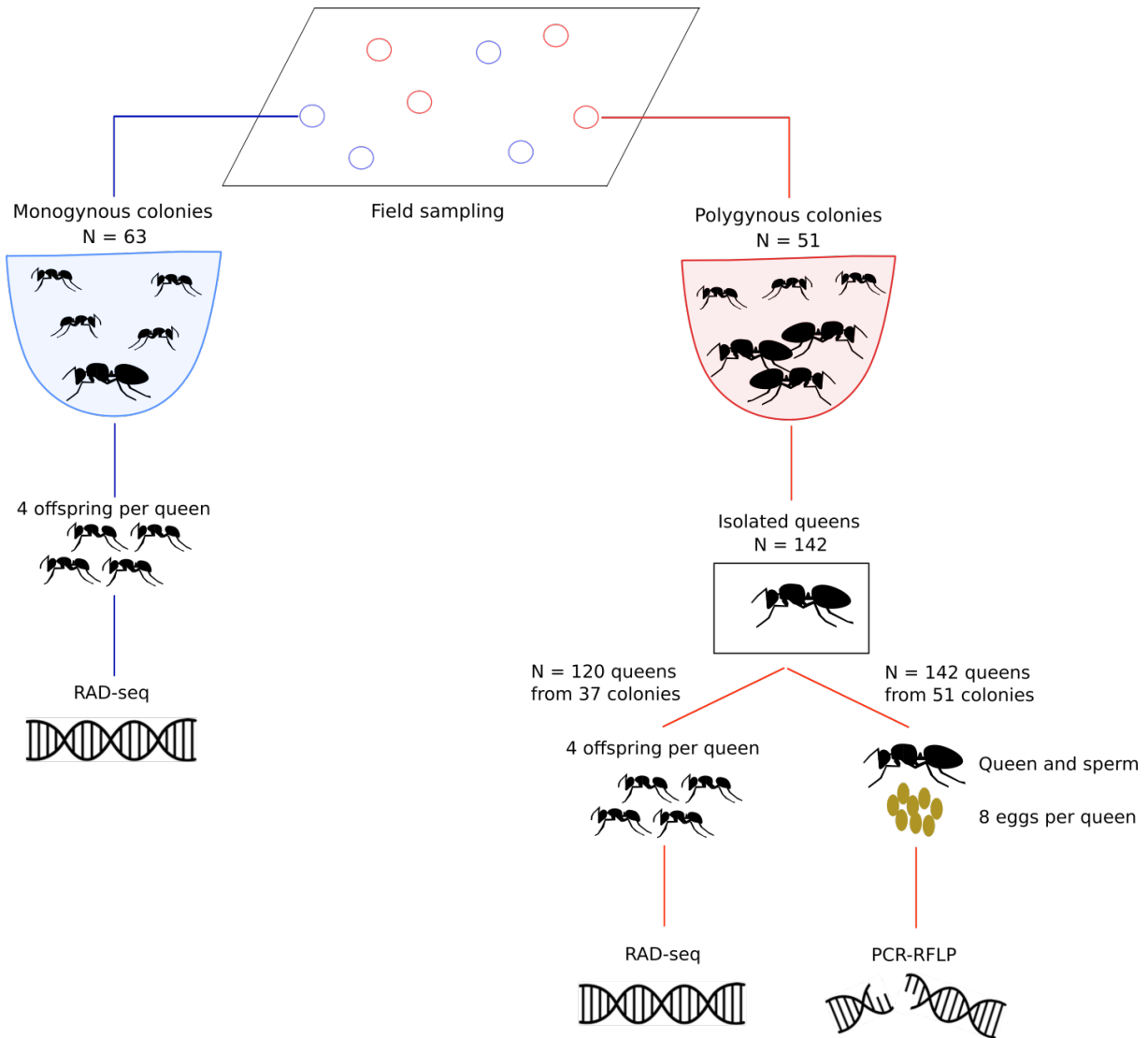


Figure S2. Scheme of the sampling and genotyping strategy.