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Cryptic recessive lethality of a supergene controlling social organization in ants

Pierre Blacher[1] | Ornela De Gasperin[1,2] | Guglielmo Grasso[1,3] | Solenn Sarton-Lohéac[1] | Roxane Allemann[1] | Michel Chapuisat[1]

[1]Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

[2]Red de Ecoetología, Instituto de Ecología A. C., Veracruz, Mexico

[3]University of Manchester, Manchester, UK

Correspondence
Ornela De Gasperin, Red de Ecoetología, Instituto de Ecología A. C., Xalapa, 91073, Veracruz, Mexico.
Email: ornela.gasperin@gmail.com

Pierre Blacher and Michel Chapuisat, Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland.
Email: pierre.blacher@unil.ch; michel.chapuisat@unil.ch

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Abstract

Supergenes are clusters of linked loci that control complex phenotypes, such as alternative forms of social organization in ants. Explaining the long-term maintenance of supergenes is challenging, particularly when the derived haplotype lacks homozygous lethality and causes gene drive. In the Alpine silver ant, *Formica selysi*, a large and ancient social supergene with two haplotypes, *M* and *P*, controls colony social organization. Single-queen colonies only contain *MM* females, while multiqueen colonies contain *MP* and *PP* females. The derived *P* haplotype, found only in multiqueen colonies, selfishly enhances its transmission through maternal effect killing, which could have led to its fixation. A population genetic model showed that a stable social polymorphism can only be maintained under a narrow set of conditions, which includes partial assortative mating by social form (which is known to occur in the wild), and low fitness of *PP* queens. With a combination of field and laboratory experiments, we show that the *P* haplotype has deleterious effects on female fitness. The survival rate of *PP* queens and workers was around half that of other genotypes. Moreover, *P*-carrying queens had lower fertility and fecundity compared to other queens. We discuss how cryptic lethal effects of the *P* haplotype help stabilize this ancient polymorphism.

KEYWORDS
ants, genetic load, inversions, social polymorphism, supergenes

1 | INTRODUCTION

Supergenes are clusters of linked loci segregating as single Mendelian units, without recombination (Gutiérrez-Valencia et al., 2021; Schwander et al., 2014; Thompson & Jiggins, 2014). Supergenes ensure that combinations of alleles producing adaptive multitrait phenotypes are inherited together (Gutiérrez-Valencia et al., 2021; Schwander et al., 2014; Thompson & Jiggins, 2014). Across the tree of life, supergenes control a range of discrete phenotypes (Charlesworth, 2015; Llaurens et al., 2017; Schwander et al., 2014), from sexes to colour morphs to alternative forms of social organization.

Supergenes arise when structural changes (typically chromosomal inversions, but also deletions, duplications and repeat accumulations) or epigenetic changes suppress recombination between alternative haplotypes (Faria et al., 2019; Gutiérrez-Valencia

Pierre Blacher and Ornela De Gasperin contributed equally to this work.

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et al., 2021; Kirkpatrick, 2010; Kirkpatrick & Barton, 2006; Sun et al., 2017). A derived supergene haplotype will spread in the population if it captures alleles that are under positive selection (Charlesworth, 1996; Durmaz et al., 2020; Schwander et al., 2014), and/or alleles that selfishly enhance their transmission (Avril et al., 2020; Chapuisat, 2023). The derived haplotype may increase in frequency until it reaches fixation. Alternatively, its spread can be halted by stabilizing forces, leading to a balanced polymorphism (Faria et al., 2019; Llaurens et al., 2017). Forces balancing polymorphisms include negative frequency-dependent selection (Fitzpatrick et al., 2007; Sinervo & Lively, 1996), disassortative mating (Gray & McKinnon, 2007), heterozygote advantage (overdominance; Johnston et al., 2013; Maisonneuve et al., 2020), and spatially or temporally varying selection (Mojica et al., 2012).

Many derived supergene haplotypes are recessive lethal (Jay et al., 2021; Klein et al., 1984; Küpper et al., 2016; Russell, 1979). This happens because derived haplotypes tend to capture and accumulate deleterious mutations, due to processes such as gene disruption at inversion breakpoints, genetic drift, stronger effects of linked selection (genetic hitchhiking, background selection; Charlesworth & Charlesworth, 2000), and protection from purifying selection in heterozygotes (Charlesworth & Charlesworth, 2000; Faria et al., 2019; Kirkpatrick, 2010; Küpper et al., 2016; Ohta, 1971). Genetic drift is particularly strong in derived haplotypes, because they experience small effective population sizes following their formation (Charlesworth & Charlesworth, 2000). In a positive feedback loop, recessive deleterious mutations reduce the frequency of homozygotes, which further limits the occurrence of recombination and the purging of load, leading to further accumulation of load by Muller's ratchet (Bachtrog & Andolfatto, 2006; Charlesworth & Charlesworth, 2000; Dolgin & Charlesworth, 2008; Felsenstein, 1974). Hence, derived haplotypes tend to occur only in heterozygotes (Berdan et al., 2021; Charlesworth & Charlesworth, 2000; Ohta, 1971).

Recessive-lethal mutations often contribute to stabilizing supergene polymorphisms (Durmaz et al., 2020; Jay et al., 2021; Kirkpatrick & Barton, 2006; Llaurens et al., 2017), at least transitorily (Berdan et al., 2022), as they generate heterozygote advantage or (associative) overdominance (Berdan et al., 2021; Charlesworth & Charlesworth, 2018; Dobzhansky, 1970; Faria et al., 2019; Llaurens et al., 2017). However, when homozygotes for both haplotypes are viable, as occurs in Alpine silver ants (*Formica selysi;* Purcell et al., 2014), stick insects (*Timema cristinae;* Lindtke et al., 2017), Atlantic cod species (Berg et al., 2016) and zebra finches (Knief et al., 2017), the factors preventing the derived haplotype from going to fixation are less clear.

In the Alpine silver ant, a large supergene with two haplotypes controls social organization (Avril, Purcell, et al., 2019; Brelsford et al., 2020; Purcell et al., 2014). The ancestral social supergene haplotype (*M*) is associated with single-queen (monogyne) colonies, while the derived haplotype (*P*) is associated with multiqueen (polygyne) colonies (Brelsford et al., 2020; Chapuisat, 2023). These haplotypes were previously referred to as *Sm* and *Sp*, respectively, but we opt for the novel notation, *M* and *P*, for simplicity (Tafreshi et al., 2022). All females living in single-queen colonies carry two copies of the *M* haplotype, whereas all females living in multiqueen colonies carry at least one copy of the *P* haplotype (Avril, Purcell, et al., 2019; Purcell et al., 2014). The *P* haplotype, which contains three large inversions, is viable in the homozygous state, as *PP* queens and workers are frequent in field colonies of *F. selysi* (Avril, Purcell, et al., 2019; Purcell et al., 2014). Yet all well-sampled *F. selysi* populations contain both types of colony social structures (Chapuisat et al., 2004; Purcell et al., 2015), and the genetic polymorphism has persisted for about 30 million years, since the origin of the genus *Formica* (Brelsford et al., 2020; Purcell et al., 2021). This indicates that strong and persistent forces balance the polymorphism.

According to a recent population genetic model, the *F. selysi* social polymorphism can be evolutionary stable under a narrow set of parameters (Tafreshi et al., 2022). This is because the derived *P* haplotype is a selfish genetic element that distorts the laws of Mendelian inheritance by causing maternal-effect killing: empirical data show that any brood from heterozygous queens that does not inherit *P* stops developing before the larval stage (Avril et al., 2020). According to this model, as long as mating occurs randomly with respect to social forms, the drive caused by *P* prevents *M* from invading a population where *P* is fixed, without any internal polymorphic equilibrium (Tafreshi et al., 2022). However, a stable polymorphic equilibrium can be reached when high rates of assortative mating by social form are combined with large fitness differences among supergene genotypes, including lower fitness of *PP* homozygotes compared to *MP* heterozygotes (Tafreshi et al., 2022). Partial assortative mating by social form is well documented in this system (Avril, Purcell, et al., 2019; Blacher et al., 2021; Fontcuberta et al., 2021), but whether *P* induces detrimental effects in *PP* females remains unknown.

Here, we carried out a series of laboratory and field experiments to investigate whether the *P* haplotype of the social supergene has cryptic deleterious effects in females, particularly when they are homozygous, which is required to balance the polymorphism according to theory (Tafreshi et al., 2022). First, we compared the survival, fecundity (probability of producing a brood) and fertility (size of the brood produced) of queens with alternative genotypes at the supergene, as well as the survival of their worker daughters (all these factors influence a queen's lifetime reproductive success). For this, we measured the proportion of *PP* and *MP* workers over development from egg to adult, in both laboratory and field colonies. These experiments revealed a negative effect of the *P* haplotype on female fitness, particularly homozygous females. We discuss how this cryptic recessive lethality, where only part of the *PP* individuals is viable, helps stabilize the genetic polymorphism at this social supergene.

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2 | MATERIALS AND METHODS

2.1 | Experimental design and general methods

2.1.1 | Survival and reproductive success of queens with alternative supergene genotypes

We measured the survival and brood production of 818 *MM*, 171 *MP* and 97 *PP* young queens in controlled laboratory conditions (Table S1; Figure 1a). In addition, we isolated part of their brood and compared the survival of 182 *MM* workers, 250 *MP* workers and 24 *PP* workers (Table S2; Figure 1a).

2.1.2 | Survival of *PP* and *MP* workers over development

Within the polygyne social form, we evaluated whether the supergene genotype of workers affects worker survival over development, and whether *PP* lethality reduces the frequency of *PP* workers in multiqueen colonies (Figure 1b). To do this, we compared the proportion of *PP* and *MP* genotypes in workers at the egg, pupa and adult stages. First, we genotyped workers produced within laboratory colonies established by one *MP* queen mated to a *P* male. These queens produce *PP* and *MP* eggs in equal proportions (Avril et al., 2020), and the expected ratio of *PP* to *MP* workers is therefore 1:1 if the two genotypes confer equal viability. Second, we measured worker genotype frequencies in multiqueen colonies in the field, to infer worker survival in natural conditions. All manipulations and observations were done blind with respect to individual genotypes.

2.2 | Detailed procedures

2.2.1 | Survival and reproductive success of queens with alternative supergene genotypes

We collected *F. selysi* ants from two populations in Switzerland (Finges: 46.3138°N, 7.6012°E, 400 m a.s.l.; Derborence: 46.2806°N, 7.2157°E, 1450 m a.s.l.). In summer 2017 and 2019, we sampled sexual pupae and workers from marked colonies of known social form, determined by genotyping three workers per colony, following Fontcuberta et al. (2021). We kept colony fragments in plastic boxes (15.5 × 13.5 × 5.5 cm) lined with fluon, with a glass tube (length = 16 cm; ø = 5 mm) one-third filled with water as a nest site, and with food in the form of egg and apple jelly ad libitum, as described in De Gasperin et al. (2020). We separated young queens from nestmate males twice a week, to prevent them from mating, as we wanted to control the genotype of the resulting offspring.

To measure the fertility of queens, we let queens mate with non-nestmate males from the same population (to avoid possible incompatibilities), collected from either single-queen or multiqueen colonies (*M* and *P* males, respectively, as male ants are haploid). Queens and males were placed inside plastic boxes

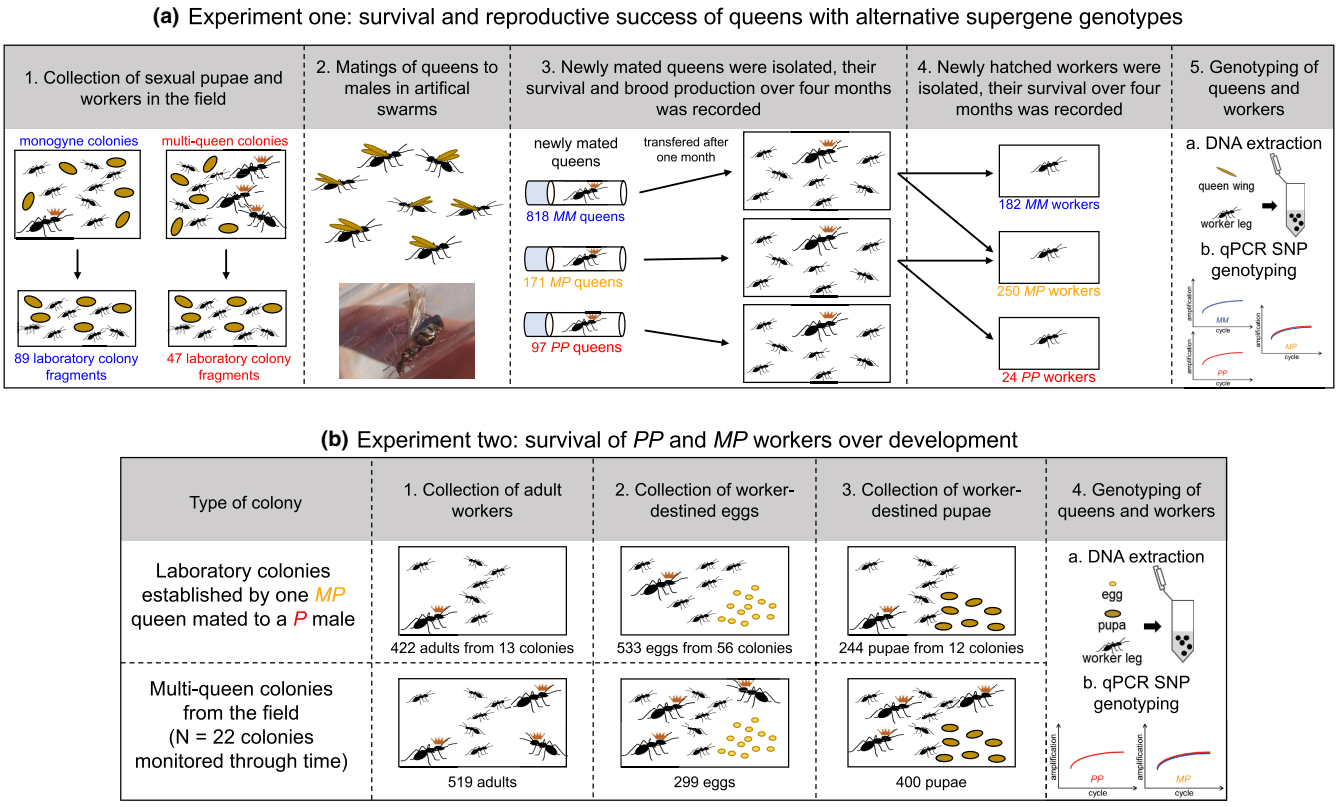

FIGURE 1 Schemes representing the two experimental designs

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(26.5×42×20 cm) with meshed lids, in conditions eliciting mating (in the mornings under direct sunlight, with males and females in proximity; Avril, Zahnd, et al., 2019; Blacher et al., 2021; De Gasperin et al., 2020). We collected any pair that mated and housed each queen inside a glass tube, as described above. After ~1 month, we transferred each queen alongside her brood to a plastic box (10.5×13.5×5.5 cm), in which they remained for the rest of the experiment. After ~4 months, we recorded queen survival and the number of workers they had produced. Queens that mated in 2017 underwent an additional experiment, but in the present study, we only used survival data before the start of the additional experiment reported in De Gasperin et al. (2020). In addition, from July to September 2019, we collected and isolated newly hatched workers (identified by their pale colour at hatching) from these laboratory colonies. We assessed worker survival after ~4 months. All individuals were kept at 25±3°C, with 70% humidity, under a 12:12-h light–dark cycle, and with water and food ad libitum. We determined the supergene genotype of each individual by analysing DNA extracted from one leg (workers) or wing (queens), following Fontcuberta et al. (2021). Briefly, we used a quantitative polymerase chain reaction (qPCR) assay based on TaqMan probes specific to each supergene haplotype and differing at three diagnostic single nucleotide polymorphisms (SNPs) within a conserved region of the supergene (Fontcuberta et al., 2021). All genotyping was done following this method.

2.2.2 | Survival of *PP* and *MP* workers over development

We compared the proportion of *PP* and *MP* eggs, pupae and adult workers produced by *MP* queens mated to *P* males and reproducing individually in laboratory colonies. The data set comprised eggs from 56 queens collected from 27 mature colonies and established in laboratory colonies in 2016 (mean±SE number of eggs genotyped per queen = 9.5±0.6; Avril, Purcell, et al., 2019), as well as pupae (mean±SE number of pupae genotyped per queen = 20.3±3.9) and adult workers (mean±SE number of workers genotyped per queen = 32.5±2.5) from 14 queens established in laboratory colonies in 2017 (see above). These colonies do not produce queens nor males in the laboratory (personal observations).

In spring, 2018, we identified wild multiqueen colonies in the population Finges, by genotyping three workers per colony. We collected eggs and adult workers from each colony. Adult workers collected in spring were born in previous year(s) and were therefore the oldest workers in our sample. One month later, when the brood laid in spring had reached the pupal stage, we collected worker pupae within the same colonies. We restricted the analysis to colonies from which we obtained all three sampled brood stages (i.e., eggs, pupae and adult workers). We monitored colonies throughout the season to assess if any queen and male brood was produced in these colonies. Sexual brood is easily recognizable by its larger size, in comparison to worker brood. Colonies producing queens and/or males were excluded, because the inclusion of haploid brood would bias the estimate of homozygous and heterozygous genotypes. We thus determined the supergene genotypes of worker-destined eggs, pupae and adults from 22 colonies (mean±SE number of eggs, pupae and adult workers genotyped per colony = 13.6±1.2, 18.2±0.5 and 23.6±0.9, respectively).

2.3 | Statistical analyses

2.3.1 | General procedures

All analyses were carried out in R version 4.1.1 (R Core Team, 2021). For generalized linear mixed models (GLMMs), we used the "glmer" function (Bates et al., 2012) and evaluated model assumptions with diagnostic plots following the methods described by Zuur et al. (2009) and using the "DHARMa" package (Hartig, 2020). We removed nonsignificant interactions to evaluate main effects, when applicable. We obtained estimates with type III sum of squares for models with interactions and with type II sum of squares for models without interactions ("Anova" function; Fox et al., 2012), and estimates, standard errors (SE) and *p* values with the "summary" function for continuous variables (R Core Team, 2021) and with the "lsmeans" function for categorical variables (Lenth, 2016). Post hoc tests were conducted using the "lsmeans" function with false discovery rate-adjusted *p* values.

2.3.2 | Survival and reproductive success of queens with alternative supergene genotypes

We assessed whether the supergene genotype affected the survival of queens and workers by comparing the proportion of individuals alive at the end of the experiment. We used two GLMMs with binomial error distribution, one for queens and one for workers. For queens, we included the supergene genotype of the queen (with three levels), the supergene haplotype of her mate (with two levels) and the interaction between these factors as explanatory variables. We included the colony of origin nested within year as a random effect. We also included the population of origin as a covariable and controlled for the number of days for which survival was monitored as a continuous covariable. Because monitoring time was highly correlated with the population of origin (the low-elevation population produces sexuals earlier; $r>0.6$), we removed the population of origin from our model, and verified that this did not affect our conclusions. For workers, we included as explanatory variables the supergene genotype of the worker, the supergene genotype of her mother (*MP* or *MM* queens), their population of origin and the number of days for which the worker's survival was monitored. We also included the identity of their mother as a random effect, because we had sister-workers in the data set.

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We examined whether *PP* queens had lower reproductive output than *MP* and/or *MM* queens. For this, we compared the fecundity (proportion of surviving queens that produced at least one worker within 4 months); model (1) and fertility (number of workers produced by surviving queens with at least one worker within 4 months); model (2), of the three types of queens, using GLMMs with binomial and Poisson error distribution, respectively. In both models we included the same explanatory variables and random effects as described above, except for model 2, where we did not include the interaction between the queen's supergene genotype and her mate's supergene haplotype, due to low sample size of some crosses (very few *MP* and *PP* queens mated to *M* males for practical reasons; see Avril, Purcell, et al., 2019). In this model, we included an observation-level random effect (OLRE) to account for overdispersion (Harrison, 2014). We removed monitoring time, as it was highly correlated with the OLRE (Pearson's $r = 0.83$).

2.3.3 | Survival of *PP* and *MP* workers over development

We investigated whether the proportion of *PP* individuals decreased throughout their development in laboratory and in field multiqueen colonies with two GLMMs with binomial error distribution. In both models we included the developmental stage (egg/pupae/adult) of the worker as an explanatory variable, and the colony of origin as a random effect.

3 | RESULTS

3.1 | Survival of alternative supergene genotypes

PP females had lower survival than other females (Table 1, Figure 2a,b and 3). The survival of *PP* queens and workers was 37%–55% lower than the survival of *MM* or *MP* queens and workers. *MM* and *MP* queens had similar survival probabilities (Table 1a), but *MP* workers had lower survival than *MM* workers (Table 1b). The survival of workers did not depend on the supergene genotype of their mother (Table 1b), nor on their population of origin (Estimate [Derborence-Finges] = −0.001, SE = 0.30, $z = -0.004$, $p = .99$). The survival of queens was not affected by the supergene haplotype of their mates (interaction: $\chi^2 = 0.70$, $p = .70$; Table 1a). The survival of queens and workers was negatively correlated with the length of time their survival was monitored (Table 1).

PP and *MP* queens had lower fecundity and fertility than *MM* queens (Table 2, Figures 2c,d and 3). Fewer *PP* and *MP* queens produced a brood, and those with workers produced smaller broods than *MM* queens (Table 2). *PP* and *MP* queens were as likely to produce a brood and produced broods of similar sizes (Table 2). The

TABLE 1 Models comparing the survival of queens and of workers with alternative supergene genotypes.

(a) Queen survival				
Explanatory variables	χ^2		**df**	***p***
Queen genotype	6.75		2	.03
Male genotype	0.24		1	.62
Monitoring time	9.82		1	.001
Queen survival: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
MM–PP	0.59	0.27	2.18	.04
MP–PP	0.75	0.30	2.51	.036
MM–MP	0.16	0.23	−0.68	.49
(b) Worker survival				
Explanatory variables	χ^2		**df**	***p***
Worker's genotype	14.57		2	.0007
Mother's genotype	0.005		1	.94
Monitoring time	11.72		1	.0006
Population	0.00		1	.99
Worker survival: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
MM–PP	2.14	0.57	3.71	.0006
MP–PP	1.39	0.49	2.77	.008
MM–MP	0.75	0.29	2.63	.008

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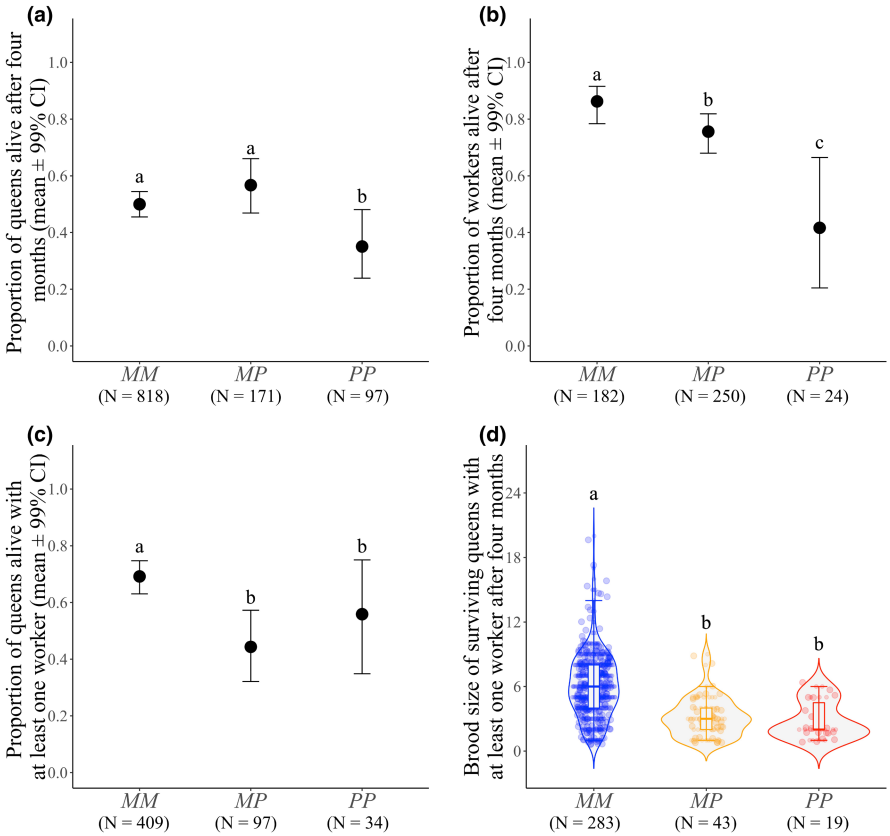

FIGURE 2 Survival, fecundity and fertility of alternative supergene genotypes. (a) Proportion of queens alive after 4 months. (b) Proportion of workers alive after 4 months. (c) Proportion of queens alive with at least one worker after 4 months. (d) Brood size (number of workers) of surviving queens with at least one worker after 4 months. The total number of individuals per genotype is displayed in parentheses (N). Different letters above error bars/scatterplots indicate significant statistical differences below the 0.05 threshold after false discovery rate adjustment for multiple comparisons. In (a), (b), and (c), error bars represent 99% CI.

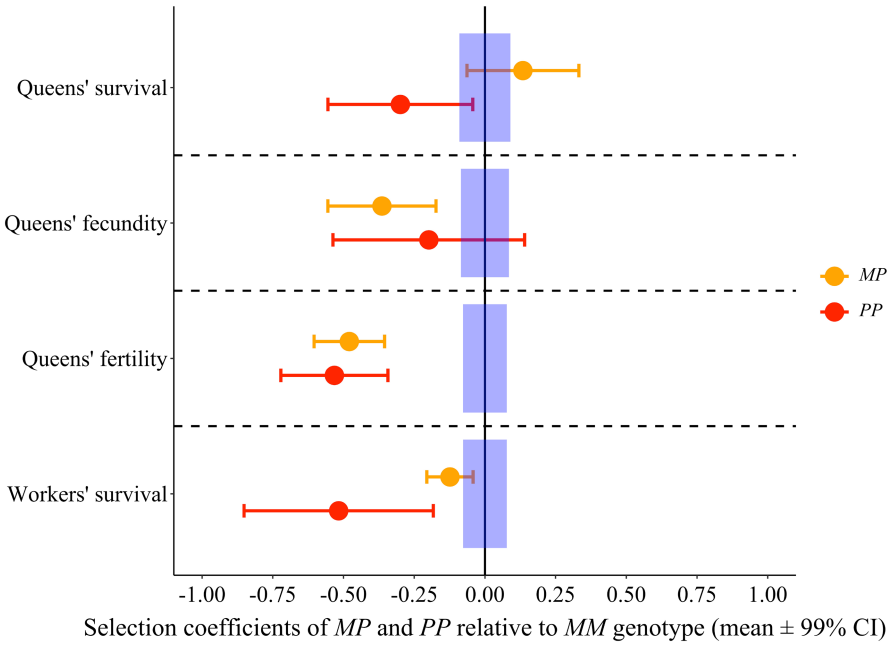

FIGURE 3 Survival, fecundity and fertility of alternative supergene genotypes. Deleterious effects linked to the *P* supergene haplotype reduce the survival, fecundity and fertility of queens, as well as the survival of workers. Dots and lines correspond to the selection coefficient for the *MP* (orange) and *PP* (red) genotypes. Selection coefficients were calculated relative to the mean value of the ancestral *MM* genotype, with light blue rectangles representing the 99% confidence interval (CI) for the selection coefficient of the *MM* genotype. Queens' fecundity refers to the probability that surviving queens produced a brood, and queens' fertility refers to the size of their brood (excluding queens that did not produce a brood).

probability that queens produced a brood was not affected by the supergene haplotype of their mates (interaction: $\chi^2 = 2.09$, $p = .35$; Table 2a). Queens mated to *P* males produced marginally significantly smaller broods than queens mated to *M* males (Table 2b; Estimate [*M*-*P* male] = 0.11, SE = 0.06, $z = 1.93$, $p = .05$). Queens from the population Derborence produced smaller broods than queens from Finges (Table 2b; Estimate [Derborence-Finges] = −0.20, SE = 0.09, $z = -2.11$, $p = .035$).

3.2 | Survival of *PP* and *MP* workers over development

PP workers had lower survival probabilities than *MP* workers (Table 3, Figure 4). As a result, the proportion of *PP* to *MP* workers decreased over development in laboratory (Table 3a, Figure 3a) and field colonies (Table 3b, Figure 4b). In laboratory colonies established by one *MP* queen mated to a *P* male, the proportion of *PP* to

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TABLE 2 Models comparing the fecundity and fertility of queens with alternative supergene genotypes.

(a) Proportion of queens alive after 4 months with at least one worker (fecundity)				
Explanatory variables	χ^2		**df**	***p***
Queen genotype	12.93		2	.001
Male genotype	1.92		1	.166
Monitoring time	37.97		1	<.001
Queen fecundity: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
MM–PP	0.99	0.45	2.23	.038
MP–PP	−0.09	0.48	−0.19	.85
MM–MP	1.08	0.33	3.30	.003
(b) Size of the brood produced by queens alive after 4 months with at least one worker (fertility)				
Explanatory variables	χ^2		**df**	***p***
Queen genotype	42.46		2	<.001
Male genotype	3.74		1	.05
Population	4.45		1	.035
Queen fertility: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
MM–PP	0.75	0.16	4.67	<.001
MP–PP	0.16	0.18	0.87	.38
MM–MP	0.59	0.11	5.16	<.001

MP workers dropped from 47% among eggs, which is close to the expected Mendelian proportion, to 31% among pupae, to only 23% among adults (Figure 4a). As wild polygyne colonies are formed by multiple reproducing *MP* and *PP* queens, which are mated primarily to *P* males, we did not expect a 1:1 ratio of *MP* and *PP* broods, as we did in our laboratory colonies. In field colonies, the proportion of *PP* workers dropped from 75% among eggs, to 50% among pupae, to only 34% among adults (Table 3b, Figure 4b).

4 | DISCUSSION

Supergenes control discrete phenotypes in a wide range of organisms (Berg et al., 2016; Chapuisat, 2023; Koch et al., 2021; Llaurens et al., 2017; Thompson & Jiggins, 2014). These polymorphisms are protected by heterozygote advantage if the derived haplotype is under positive selection in heterozygotes, but harbours recessive-lethal mutations (Brinkhurst, 1959; Hallar et al., 2007; Klein et al., 1984; Küpper et al., 2016; Russell, 1979). However, in the supergene controlling social organization in *F. selysi*, empirical data revealed that the derived haplotype distorts transmission, but is not a homozygous lethal, as *PP* individuals are common in multiqueen colonies (Avril, Purcell, et al., 2019; Purcell et al., 2014). A recent population genetic model showed that when mating is random with respect to social form, the *F. selysi* social polymorphism is never stable (Tafreshi et al., 2022). In particular, the driving *P* haplotype often goes to fixation, at which point it cannot be invaded by the ancestral *M* haplotype. According to this model, a stable polymorphism is only reached under restrictive conditions that include high but incomplete assortative mating by social form and lower fitness of the *PP* genotype, compared to those of the *MP* and *MM* genotypes (Tafreshi et al., 2022). Here, we provide support for the second condition. In laboratory and field experiments, we found that (i) *PP* females have lower survival than *MP* and *MM* females, and (ii) *P*-carrying queens have lower fertility and fecundity than other queens. Hence, the *P* haplotype has recessive lethal effects in queens, and other detrimental effects that reduce the fitness of the *PP* genotype, which contribute to stabilizing the polymorphism.

Our study provides strong evidence that the social supergene haplotype (*P*) that induces multiqueen colonies in Alpine silver ants has deleterious effects on the fitness of females. *P* is not fully lethal in homozygotes, as approximately half of the queens living in wild multiqueen colonies are *PP* (Avril, Purcell, et al., 2019; Brelsford et al., 2020; Purcell et al., 2014). Yet, over the first 4 months of their adult life, *PP* queens and workers had survival rates that were between 37% and 55% lower than those of *MP* and *MM* queens and workers. As *MP* and *PP* queens are both produced by polygyne colonies, the difference in survival of these queens shows a direct effect of the supergene on queen survival. Moreover, in field conditions, most *PP* workers died before reaching adulthood. Hence, *P* has cryptic recessive lethal effects in queens, and codominant detrimental

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effects on queen fertility and worker survival, which greatly decrease the survival of homozygotes in their early life. In addition, *P* reduces the fecundity and fertility of queens. Specifically, fewer *PP* and *MP* queens had workers than *MM* queens, and the two former had smaller broods than the latter.

TABLE 3 Models comparing the proportion of *PP* and *MP* workers over development

(a) Proportion of *PP* and *MP* workers over developmental stages in laboratory colonies				
Explanatory variable		χ^2	**df**	***p***
Developmental stage		30.76	2	<.001
Developmental stage in laboratory colonies: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
Pupae–eggs	−0.63	0.21	−3.03	.004
Adults–eggs	−1.05	0.19	−5.55	<.0001
Adults–pupae	−0.42	0.19	2.19	.029
(b) Proportion of *PP* and *MP* workers over developmental stages in field colonies				
Explanatory variable		χ^2	**df**	***p***
Developmental stage		120.2	2	<.0001
Developmental stage in wild colonies: Post hoc comparisons				
Comparison	**Estimate**	**SE**	***z***	***p***
Pupae–eggs	−1.21	0.18	−6.53	<.0001
Adults–eggs	−2.01	0.18	−10.93	<.0001
Adults–pupae	−0.81	0.15	−5.27	<.0001

The high mortality of *PP* females and the lower fertility of *P*-carrying females suggests that the *P* haplotype contains deleterious mutations. This was somewhat unexpected, as deleterious mutations might have been purged by recombination in viable *PP* homozygotes. However, deleterious mutations could have been fixed within the newly derived inversions, due to disruption of genes at inversion breakpoints, linked selection (genetic hitchhiking, background selection) and drift when the effective population size of the derived haplotype was small (Berdan et al., 2021; Charlesworth & Charlesworth, 2000; Faria et al., 2019; Jay et al., 2021; Kirkpatrick, 2010; Navarro et al., 2000; Villoutreix et al., 2021). Furthermore, linkage disequilibrium decreases the efficiency of recombination at purging load (Jay et al., 2021). Further molecular characterization of the social supergene will reveal how the *P* haplotype evolved (Brelsford et al., 2020; Purcell et al., 2021), and whether it contains deleterious mutations in coding regions (e.g., missense mutations, stop mutations, inversion breakpoints) or control regions. In *F. selysi*, we have already found evidence that the *P* haplotype expanded in size, and accumulated gene duplicates and transposable elements (Chapuisat, 2022). However, demonstrating causal lethal or detrimental effects of specific mutations will be a substantial challenge, as the *P* haplotype is 13.8 Mb long, contains 748 genes, and differs from the *M* haplotype in many ways (Chapuisat, 2022).

The fact that half of the queens in wild multiqueen colonies have the *PP* genotype (Avril, Purcell, et al., 2019) may appear surprising at first, given the low survival associated with this genotype. However,

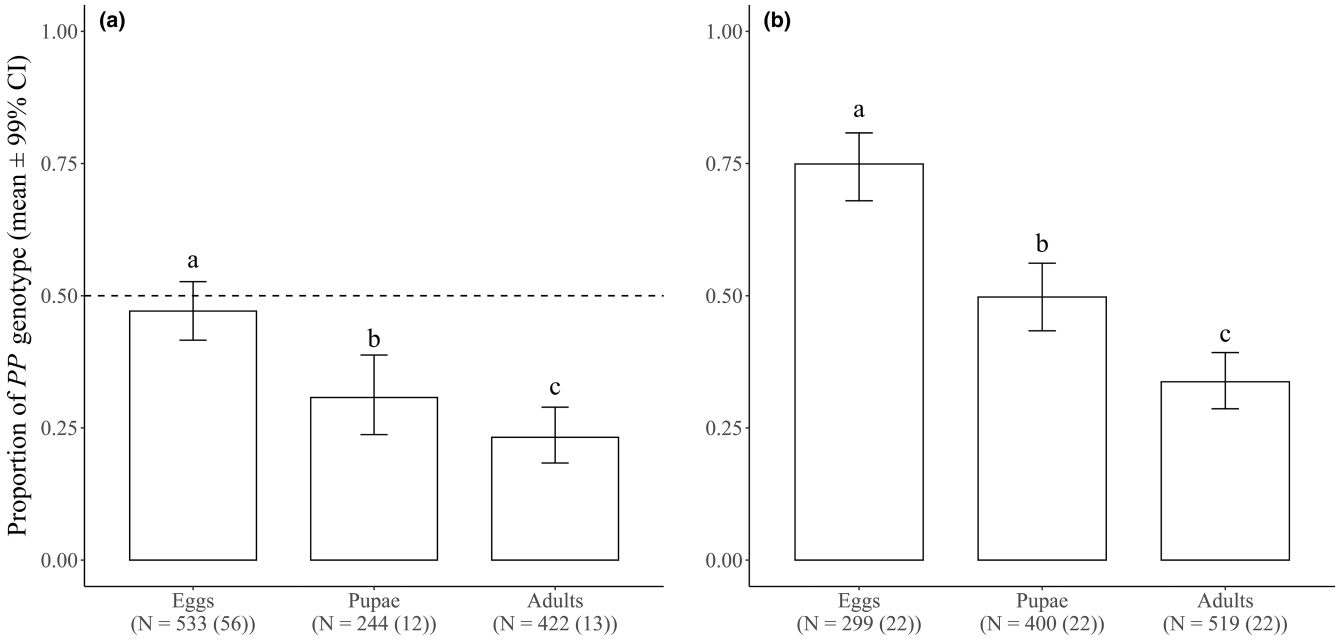

FIGURE 4 Survival of *PP* and *MP* workers over development. The proportion of *PP* to *MP* genotypes decreased throughout development, both in laboratory colonies established by one *MP* queen mated to a *P* male (a) and in multiqueen colonies from the field (b), which reveals that a large proportion of *PP* eggs fail to develop into adults. The horizontal dashed line in panel (a) shows the proportion of *PP* individuals expected under the 1:1 Mendelian ratio. The number of individuals alive (N) and the number of colonies (in parentheses) are displayed below the bars. Different letters above bars indicate significant statistical differences below the .05 threshold after false discovery rate adjustment for multiple comparisons. Whiskers represent 99% confidence intervals (CI).

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this apparent paradox is easily explained by the mating pattern of polygyne queens. Most queens in multiqueen colonies mate assortatively with respect to social form. Specifically, 77.1% of polygyne queens are mated to *P* males (Avril, Purcell, et al., 2019; Blacher et al., 2021; Fontcuberta et al., 2021). Based on this observed rate of assortative mating, the proportion of *PP* queens is expected to be as high as 67% in multiqueen colonies, if *PP* and *MP* queens had similar fitness (Figure S1). Yet the observed proportion of mature *PP* queens in multiqueen colonies is only 48.7% (Avril, Purcell, et al., 2019). According to our simulations (see Supporting Information), this proportion is expected when the fitness of *PP* queens is 40% lower than the fitness of *MP* queens (Figure S1). This latter estimate closely matches our empirical estimate. Indeed, the survival of *PP* queens was 37.5% lower than the survival of *MP* queens (in our experiment, 56% of *MP* queens survived, while 35% of *PP* queens survived). In short, high levels of assortative mating suffice to explain the high proportion of *PP* queens observed in wild polygyne colonies, despite their reduced survival.

In Alpine silver ants, the drive of the *P* supergene haplotype destabilizes the polymorphism, but a stable internal polymorphic equilibrium can be reached when high levels of assortative mating are combined with large fitness differences between supergene genotypes (Tafreshi et al., 2022). High levels of assortative mating within social forms have been repeatedly reported in this system (Avril, Purcell, et al., 2019; Blacher et al., 2021; Fontcuberta et al., 2021). Our results provide empirical support for the second theoretical prediction, as we uncovered that the fitness of *PP* queens is lower than that of *MP* queens, one of the conditions leading to a stable polymorphism (Tafreshi et al., 2022). However, the lifetime fitness of each of the three supergene genotypes is difficult to estimate, because the ant social forms differ in many life-history traits, including dispersal and colony founding (Fontcuberta et al., 2021; Rosset & Chapuisat, 2007; Tafreshi et al., 2022). Moreover, colony-level selection and various forms of density- and frequency-dependent selection can help stabilize the polymorphism (Zahnd et al., 2021). Despite these complications, cryptic recessive lethal effects in queens, and other deleterious effects of the driving haplotype, probably contribute to balancing this ancient social polymorphism.

Social supergenes have evolved independently multiple times in ants, and these supergenes show striking similarities. There are five ant social supergenes described so far (i.e., in *F. selysi*, *Solenopsis invicta*, *Leptothorax acervorum*, *Cataglyphis niger* and *Pogonomyrmex californicus*; reviewed in Kay et al., 2022), all of which induce alternative forms of colony social organization. All sequenced supergenes are large, being 8–12 Mb long and formed by 400–750 genes. The two best studied supergenes, in *F. selysi* and *S. invicta*, present multiple inversions, display selfish effects (maternal-effect killing in the former, greenbeard in the latter) and are associated with high levels of nonrandom mating with respect to supergene genotypes (reviewed in Chapuisat, 2023; Kay et al., 2022). Our results show another important and striking similarity between these social supergenes: deleterious effects of the derived, polygyny-inducing haplotype. Specifically, in fire ants, queens homozygous for the polygyny-inducing haplotype die before reaching maturity (Gotzek & Ross, 2007), and recessive lethality seems to also occur in *C. niger* (Kay et al., 2022). Our results indicate that recessive lethality of the derived, polygyny-inducing haplotypes is common in social supergenes, a characteristic that contributes to preventing polygyne colonies from going to fixation.

In conclusion, we found that the derived haplotype of the social supergene of Alpine silver ants reduces the survival of *PP* females and decreases their frequency within multiqueen colonies. Our results provide empirical support for the theoretical prediction that *PP* queens must have lower fitness than other queens to balance this ancient polymorphism (Tafreshi et al., 2022). More generally, our results add to the consensus that nonrecombining genomic regions commonly cause deleterious effects, and that recessive lethality plays a major role in the evolutionary dynamics of supergenes, even when some homozygotes are viable.

AUTHOR CONTRIBUTIONS

Designed research: Pierre Blacher, Ornela De Gasperin and Michel Chapuisat. Performed research: Pierre Blacher, Ornela De Gasperin, Guglielmo Grasso, Solenn Sarton-Lohéac and Roxane Allemann. Analysed data: Pierre Blacher, Ornela De Gasperin and Michel Chapuisat. Wrote the paper: Pierre Blacher, Ornela De Gasperin and Michel Chapuisat.

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CONFLICT OF INTEREST

We declare we have no conflicts of interests.

DATA AVAILABILITY STATEMENT

Supporting data is available in Dryad Digital Repository https://doi.org/10.5061/dryad.dz08kps1n (Blacher et al., 2022).

ORCID

Pierre Blacher https://orcid.org/0000-0002-4255-4277
Ornela De Gasperin https://orcid.org/0000-0003-3415-2072
Michel Chapuisat https://orcid.org/0000-0001-7207-199X

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