ORIGINAL ARTICLE

THE EFFECTS OF KIN SELECTION ON RATES OF MOLECULAR EVOLUTION IN SOCIAL INSECTS

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The evolution of sociality represented a major transition point in biological history. The most advanced societies, such as those displayed by social insects, consist of reproductive and nonreproductive castes. The caste system fundamentally affects the way natural selection operates. Specifically, selection acts directly on reproductive castes, such as queens, but only indirectly through the process of kin selection on nonreproductive castes, such as workers. In this study, we present theoretical analyses to determine the rate of substitution at loci expressed exclusively in the queen or worker castes. We show that the rate of substitution is the same for queen- and worker-selected loci when the queen is singly mated. In contrast, when a queen is multiply mated, queen-selected loci show higher rates of substitution for adaptive alleles and lower rates of substitution for deleterious alleles than worker-selected loci. We compare our theoretical expectations to previously obtained genomic data from the honeybee, *Apis mellifera*, where queens mate multiply and the fire ant, *Solenopsis invicta*, where queens mate singly and find that rates of evolution of queen- and worker-selected loci are consistent with our predictions. Overall, our research tests theoretical expectations using empirically obtained genomic data to better understand the evolution of advanced societies.

Biological complexity increased through a series of major evolutionary transitions characterized by the merging of independent biological entities into interdependent groups (Szathmary and Smith 1995). One of the most recent evolutionary transitions occurred when individually reproducing organisms came together to form integrated societies. The transition to sociality is best exemplified by the social insects, such as ants, social bees, social wasps, and termites, which display extraordinary cooperative and helping behaviors (Wilson 1971).

A defining feature of insect societies is a caste system, whereby distinct individuals specialize on completing particular tasks (Oster and Wilson 1978). The queen and male castes are generally responsible for reproduction and dispersal, whereas the worker and soldier castes specialize on foraging, defending the colony, and maintaining the nest. The evolution of the caste system has led to great ecological success (Wilson 1990) making

the transition to sociality in insects an important evolutionary innovation.

The evolution of integrated social groups, such as those displayed by social insects, has had important consequences for how natural selection operates. For example, selection can operate directly on members of the reproductive castes. Direct selection may occur if a queen, for instance, possesses a particular allele that confers greater survival or reproductive success than other alleles possessed by competing queens within the population. In this case, the beneficial allele will increase in frequency in the population because the queen that possesses the focal allele produces more offspring than queens that do not.

In contrast, selection may operate indirectly on the nonreproductive castes. Indirect selection may occur when a worker, for instance, possesses an allele that improves foraging efficiency that, therefore, allows the colony to produce more new reproductives.

Under this scenario, the worker never directly enjoys greater reproductive success. However, the allele still increases in frequency in the population because the reproductive individuals within the colony are related to the worker and will transmit the focal allele to future generations. Selection thus increases the frequency of the allele because of the positive effects that the allele has on the relatives of the worker. This process, more commonly known as kin selection, is responsible for many of the remarkable behaviors displayed by social insects (Bourke and Franks 1995; Queller and Strassmann 1998; Abbot et al. 2011). The interplay between kin and direct selection has made social insects fascinating and important subjects for understanding how natural selection operates (Frank 1998; Keller 1999).

This study presents results from a simple, one locus model to determine the probability of fixation for a newly arising allele that experiences direct selection through queens or kin selection through workers. The study is motivated by new data arising from genomic studies in social insects that may provide opportunities to test kin selection theory. Such data provide evidence of genes expressed predominantly, or exclusively, in certain castes (Sumner 2006; Goodisman et al. 2008; Smith et al. 2008) suggesting caste-specific function. These loci are strong candidates for being under different types of selection: direct selection in reproductive queens or males and kin selection in workers or soldiers.

Our analysis builds on recent work by Linksvayer and Wade (2009) who examined the probability of fixation of alleles with direct effects, indirect maternal effects and indirect sib-social effects. However, there are several differences. The main distinction is that we consider sex-limited selection for both direct (queen) and indirect (worker) selection, both of which act through females only. We concentrate on these two types of selection primarily to model social hymenopteran insects (ants, social bees, and social wasps), which are among the best studied social taxa. Hymenopteran insects display a haplodiploid genetic system. More importantly, hymenopteran insect societies are female-dominated and colony fitness (the number of reproductives produced) is likely determined largely by traits of queens and workers (Michener 1974; Ross and Matthews 1991; Bourke and Franks 1995). Further, we explore any degree of dominance for an introduced allele, rather than assuming that alleles act additively.

We find that with sex-limited selection, direct and kin selection are equally effective when queens are singly mated, and we expect no difference in rates of molecular evolution of queen versus worker-expressed loci. This result is in sharp contrast to the situation in which fitness traits are not sex limited (Linksvayer and Wade 2009). In large populations, when queens are multiply mated, adaptive alleles experiencing direct selection in queens are twice as likely to fix as adaptive alleles experiencing kin selection in workers. Further, queen-expressed maladaptive alleles are

less likely to fix than worker-expressed alleles when the queen is multiply mated.

Finally, we apply our theoretical framework to recently obtained empirical data in two hymenopteran social insects. We specifically examine rates of evolution of genes putatively under direct selection in queens and indirect selection in workers in species that vary in queen mate number. We find evidence for a match between theory and data and discuss how empirical data derived from genomic studies can now be used to test kin selection theory in social insects.

Models

We consider two simple models of evolution, which we term queen-selection and worker-selection. In both, we assume that all fitness differences are due to variation at a single locus, the *A* locus, which is initially fixed in the population. We further assume that mating is random and that all queens mate the same number of times, with the number of matings being 1, 2 or many, depending on the case. We first assume an infinite population size in order to determine the change in allele frequency for a newly introduced allele. Then, we use this deterministic change in allele frequency to estimate the probability of fixation of an allele in a population of size *N* using Kimura's (1962) extension of Haldane's (1927) result. We analyze the models under both haplodiploidy (males haploid, females diploid) and diploidy (both sexes diploid).

We assume that colonies consist of a single queen and her daughter workers. In addition, all colonies in a population produce reproductive queens and males at the same time and generations do not overlap. The fitness of a colony is the expected number of male and female reproductives that the colony produces. Colony fitness is equivalent to the direct fitness of the queen and of her mate(s). With multiple mating (polyandry), the direct fitness of each of the queen's mates is his contribution to the reproductives, which we assume is unaffected by his genotype.

In the queen-selection model, we assume that variation at the fitness locus affects queen/colony fitness directly and independently of the genotypes of the workers (Table 1). In contrast, the genotype at the focal locus has no fitness effect in males. The fitness of a colony is thus completely determined by the genotype of the single founding queen and is mathematically equivalent to a maternal effect as modeled by Linksvayer and Wade (2009). This locus might affect a queen's ability to choose a nest site, dig a hole in the substrate, efficiently utilize energy reserves, defend against pathogens or predators, or something similar.

In the worker-selection model, we assume that variation at the focal locus affects queen/colony fitness through its effects on the phenotype of individual workers. We assume that the fitness of a colony is the result of the average phenotype of its workers

(Table 1) and does not directly depend on the genotype of the founding queen or the males to which the queen mates (though the parental genotypes affect the workers' genotypes). Variation at the fitness locus might affect worker phenotypes such as foraging ability, defensive ability, resistance to pathogens and predators, competitive ability, or something similar.

For each model, we examined the fate of a new allele if queens within the population mate singly, mate doubly, or mate many times. For each of the three scenarios, we derived the recursion equations in genotype frequencies (*AA*, *Aa*, and *aa* in females and *A* and *a* in males under haplodiploidy). We then performed a stability analysis for the introduction of a newly introduced allele (*a*) into a population initially fixed for the *A* allele. The stability analysis involved calculating the Jacobian matrix and determining its eigenvalues at the *A* fixation (Edelstein-Keshet 1989). The largest eigenvalue, λ, gives the rate of increase of the newly introduced allele, which we used to calculate the change in allele frequency, M. The allele frequency in the next generation, $q(t+1)$ is:

$$q(t + 1) = \lambda q(t). \tag{1}$$

Thus, the change in allele frequency is

$$M = q(t + 1) - q(t) = (\lambda - 1)q(t). \tag{2}$$

The change in allele frequency was then used to calculate the probability of fixation of the allele. Kimura (1962) showed that the probability of fixation of an allele with initial frequency q, $u(q)$, is:

$$u(q) = \frac{\int_0^q G(x)dx}{\int_0^1 G(x)dx}, \tag{3}$$

where

$$G(x) = e^{-\int \frac{2M}{V} dx}. \tag{4}$$

V is the variance in the per-generation change in allele frequency, when the allele frequency is q. The variance in allele frequency change is $pq/(2N)$ for diploids (Kimura 1962) and $2pq/(3N)$ for haplodiploids (Linksvayer and Wade 2009), where $p = 1 - q$. When a is rare, these expressions simplify to $q/(2N)$ and $2q/(3N)$, respectively. The type of selection acting on the locus does not alter V. Putting the expressions for M and V into (4) gives:

$$G_{\mathrm{DD}}(x) = e^{-\int 4N(\lambda-1)dx} = e^{-4N(\lambda-1)x}, \tag{5}$$

and

$$G_{\mathrm{HD}}(x) = e^{-\int 3N(\lambda-1)dx} = e^{-3N(\lambda-1)x} \tag{6}$$

Table 1. Colony fitness under queen-selection and worker-selection, with 1, 2, or many queen matings. Parameters satisfy $0 \le h \le 1$ and $s \ge -1$. When $h = \frac{1}{2}$ a mutation exhibits an additive effect on fitness. When $s < 0$ a mutation is deleterious and when $s > 0$ it is beneficial. (A) Haplodiploids (haploid males, diploid females). p_m and q_m are the respective frequencies of A and a males. (B) Diploids. f_{AA}, f_{Aa}, and f_{aa} are the respective frequencies of *AA*, *Aa*, and *aa* males.

A. Haploid males

Selection	Number of matings	Queen	Male(s)	Colony fitness
Queen	Any	*AA*	Any	1
		Aa	Any	$1 + hs$
		aa	Any	$1 + s$
Worker	1	*AA*	*A*	1
			a	$1 + hs$
		Aa	*A*	$1 + \frac{1}{2} hs$
			a	$1 + \frac{1}{2} hs + \frac{1}{2} s$
		aa	*A*	$1 + hs$
			a	$1 + s$
	2	*AA*	Both *A*	1
			1 *A*, 1 *a*	$1 + \frac{1}{2} hs$
			Both *a*	$1 + hs$
		Aa	Both *A*	$1 + \frac{1}{2} hs$
			1 *A*, 1 *a*	$1 + \frac{1}{2} hs + \frac{1}{4} s$
			Both *a*	$1 + \frac{1}{2} hs + \frac{1}{2} s$
		aa	Both *A*	$1 + hs$
			1 *A*, 1 *a*	$1 + \frac{1}{2} hs + \frac{1}{2} s$
			Both *a*	$1 + s$
	Many	*AA*	p_m *A*, q_m *a*	$1 + q_m s$
		Aa	p_m *A*, q_m *a*	$1 + \frac{1}{2} hs + \frac{1}{2} q_m s$
		aa	p_m *A*, q_m *a*	$1 + p_m hs + q_m s$

B. Diploid males

Selection	Number of matings	Queen	Male(s)	Colony fitness
Queen	Any	*AA*	Any	1
		Aa	Any	$1 + hs$
		aa	Any	$1 + s$
Worker	1	*AA*	*AA*	1
			Aa	$1 + \frac{1}{2}hs$
			aa	$1 + hs$
		Aa	*AA*	$1 + \frac{1}{2}hs$
			Aa	$1 + \frac{1}{2}hs + \frac{1}{4}s$
			aa	$1 + \frac{1}{2}hs + \frac{1}{2}s$
		aa	*AA*	$1 + hs$
			Aa	$1 + \frac{1}{2}hs + \frac{1}{2}s$
			aa	$1 + s$
	2	*AA*	Both *AA*	1
			1 *AA*, 1 *Aa*	$1 + \frac{1}{4}hs$
			Both *Aa*	$1 + \frac{1}{2}hs$
			1 *Aa*, 1 *aa*	$1 + \frac{3}{4}hs$
			Both *aa*	$1 + hs$
		Aa	Both *AA*	$1 + \frac{1}{2}hs$
			1 *AA*, 1 *Aa*	$1 + \frac{1}{2}hs + \frac{1}{8}s$
			Both *Aa*	$1 + \frac{1}{2}hs + \frac{1}{4}s$
			1 *Aa*, 1 *aa*	$1 + \frac{1}{2}hs + \frac{3}{8}s$
			Both *aa*	$1 + \frac{1}{2}hs + \frac{1}{2}s$
		aa	Both *AA*	$1 + hs$
			1 *AA*, 1 *Aa*	$1 + \frac{3}{4}hs + \frac{1}{4}s$
			Both *Aa*	$1 + \frac{1}{2}hs + \frac{1}{2}s$
			1 *Aa*, 1 *aa*	$1 + \frac{1}{4}hs + \frac{3}{4}s$
			Both *aa*	$1 + s$
	Many	*AA*	$f_{AA}\,AA, f_{Aa}\,Aa, f_{aa}\,aa$	$1 + (f_{aa} + \frac{1}{2}f_{Aa})hs$
		Aa	$f_{AA}\,AA, f_{Aa}\,Aa, f_{aa}\,aa$	$1 + \frac{1}{2}hs + \frac{1}{2}(f_{aa} + \frac{1}{2}f_{Aa})s$
		aa	$f_{AA}\,AA, f_{Aa}\,Aa, f_{aa}\,aa$	$1 + (f_{AA} + \frac{1}{2}f_{Aa})hs + (f_{aa} + \frac{1}{2}f_{Aa})s$

for diploids (DD) and haplodiploids (HD), respectively. Substituting (5) and (6) into (3) gives:

$$u_{DD}(q) = \frac{\int_0^q e^{-4N(\lambda-1)x}dx}{\int_0^1 e^{-4N(\lambda-1)x}dx} = \frac{1 - e^{-4N(\lambda-1)q}}{1 - e^{-4N(\lambda-1)}} \qquad (7)$$

and

$$u_{HD}(q) = \frac{\int_0^q e^{-3N(\lambda-1)x}dx}{\int_0^1 e^{-3N(\lambda-1)x}dx} = \frac{1 - e^{-3N(\lambda-1)q}}{1 - e^{-3N(\lambda-1)}} \qquad (8)$$

as the probabilities of fixation. Assuming that newly arising alleles occur as a single copy in the population, then q will be $1/(2N)$ and $2/(3N)$ in diploids and haplodiploids, respectively.

We calculated the leading eigenvalues for queen and worker selection under the three mating scenarios, and substituted these into equations (7) and (8) to determine the probability of fixation. The rate of evolution was then calculated by multiplying the probability of fixation by the rate at which new mutants arise, which is

equal to $2N\mu$ and $3/2N\mu$ for diploids and haplodiploids, respectively, where μ is the per-allele mutation rate per generation.

Finally, we addressed whether the differences in the probabilities of fixation that we obtained could be explained by differences in relatedness within colonies. In our model, we define colony fitness as the number of reproductives produced in the next generation, which is a measure of the direct fitness of the queen and her mate(s). Therefore, the relevant relatedness measures to consider are for the individual(s) expressing the fitness locus, that is either the queen or the workers, to the queen and her mate(s).

Results

The recursion equations for our models are presented in the Appendix. The stability analyses of the various cases result in the leading eigenvalues shown in Table 2. For the haplodiploid case, the leading eigenvalues simplify substantially if we assume

Table 2. Leading eigenvalue of the stability matrix and its approximation assuming equal frequencies in males and females at birth. The probabilities of fixation for an introduced allele, using the approximate eigenvalue, are shown in the fourth column. The rates of substitution (number of alleles that fix per generation) for adaptive mutations in large populations, calculated using the fixation probability, rate of mutation (μ), and the initial frequency of a new mutation, are shown in the last column. (A) Haplodiploids, where the initial frequency of a new allele is equal to $2/(3N)$. (B) Diploids, where the initial frequency of a new allele is equal to $1/(2N)$.

A. Haploid males

Model	Queen matings	Leading eigenvalue	Fixation probability	Adaptive substitution rate
Queen-selection	Any	$\frac{1}{4}(1 + hs + \sqrt{(1 + hs)(9 + hs)}) \approx 1 + \frac{2}{3}hs$	$\frac{1 - e^{-\frac{4}{3}hs}}{1 - e^{-2Nhs}}$	$2N\mu hs$
Worker-selection	1	$\frac{1}{8}(2 + hs + \sqrt{(2 + hs)(18 + 17hs)}) \approx 1 + \frac{2}{3}hs$	$\frac{1 - e^{-\frac{4}{3}hs}}{1 - e^{-2Nhs}}$	$2N\mu hs$
Worker-selection	2	$1 + \frac{1}{2}hs$	$\frac{1 - e^{-hs}}{1 - e^{-\frac{3}{2}Nhs}}$	$\frac{3}{2}N\mu hs$
Worker-selection	Many	$\frac{1}{8}(2 + hs + \sqrt{(2 + hs)(18 + hs)}) \approx 1 + \frac{1}{3}hs$	$\frac{1 - e^{-\frac{2}{3}hs}}{1 - e^{-Nhs}}$	$N\mu hs$

B. Diploid males

Model	Queen matings	Leading eigenvalue	Fixation probability	Adaptive substitution rate
Queen-selection	Any	$1 + \frac{1}{2}hs$	$\frac{1 - e^{-hs}}{1 - e^{-2Nhs}}$	$2N\mu hs$
Worker-selection	1	$1 + \frac{1}{2}hs$	$\frac{1 - e^{-hs}}{1 - e^{-2Nhs}}$	$2N\mu hs$
Worker-selection	2	$1 + \frac{3}{8}hs$	$\frac{1 - e^{-\frac{3}{4}hs}}{1 - e^{-\frac{3}{2}Nhs}}$	$\frac{3}{2}N\mu hs$
Worker-selection	many	$1 + \frac{1}{4}hs$	$\frac{1 - e^{-\frac{1}{2}hs}}{1 - e^{-Nhs}}$	$N\mu hs$

that there is no difference in allele frequency between males and females at birth. In this case, for $hs < 1$, the approximate eigenvalues are slightly larger, but within 4% of the true eigenvalues and thus the approximation is quite accurate. For adaptive alleles with very large selection coefficients, $hs > 1$, the approximation becomes less accurate, but in these scenarios, the effect of the error on the fixation probability is minimal because the probability is close to 1. For the rest of the analysis, we will use the approximate eigenvalues. Expressions for the probabilities of fixation calculated from the eigenvalues are shown in Table 2 and values for the probabilities are shown in Figures 1 and 2.

There are three major results in Table 2. First, for both diploids and haplodiploids, the probability of fixation does not differ between queen and worker selection when queens are singly mated. Second, for worker selection in both diploids and haplodiploids the probability of fixation declines with the degree of polyandry. Third, there is no difference in the rate of substitution in diploids versus haplodiploids assuming no differences in the mutation rates per allele per generation, selection coefficients, dominance coefficients, or population sizes.

We considered if the relatedness of individuals within colonies provided further insight into the probabilities of fixation (Hamilton 1972). Our interest lies in the relatedness of the focal individual(s) in our model expressing the A locus, the queen or the workers, to the queen and her mate(s) since it is these reproductive individuals who are transmitting alleles to the next generation. In

Table 3, we show b_{EQ}, the relatedness of the expresser to the queen, and b_{EM}, the relatedness of the expresser to the male(s) mated to the queen (Hamilton 1972; Michod and Anderson 1979; Queller and Goodnight 1989; Bourke and Franks 1995). These relatedness coefficients can be thought of as the probability that another individual will transmit a particular allele carried in the focal individual, relative to the frequency of that allele in the focal individual. For example, b_{EM} in haplodiploids is the probability that a father transmits an allele present in the daughter, which equals ½ (frequency of the paternal allele), times the probability that he transmits it, which equals 1 for haploid males. The frequency of the allele in daughters is ½ giving $b_{EM} = $ ½ / ½ = 1.

We used the relatedness coefficients to calculate an average genetic contribution through the queen and her mate(s), by weighing each relatedness value by the contribution of the queen and male(s) to the reproductives produced in the next generation, assuming an even sex ratio in the population and in the brood produced by a colony. In diploids, both sexes contribute equally to the next generation and so the two relatedness measures are weighted equally. In haplodiploids, queens contribute to all reproductives, and males contribute only to female reproductives and so the two relatedness measures are weighted by ⅔ and ⅓, respectively.

Comparison of Tables 2 and 3 indicates that the mean relatedness perfectly predicts the eigenvalues associated with the introduction of a new allele. In fact, the adaptive substitution
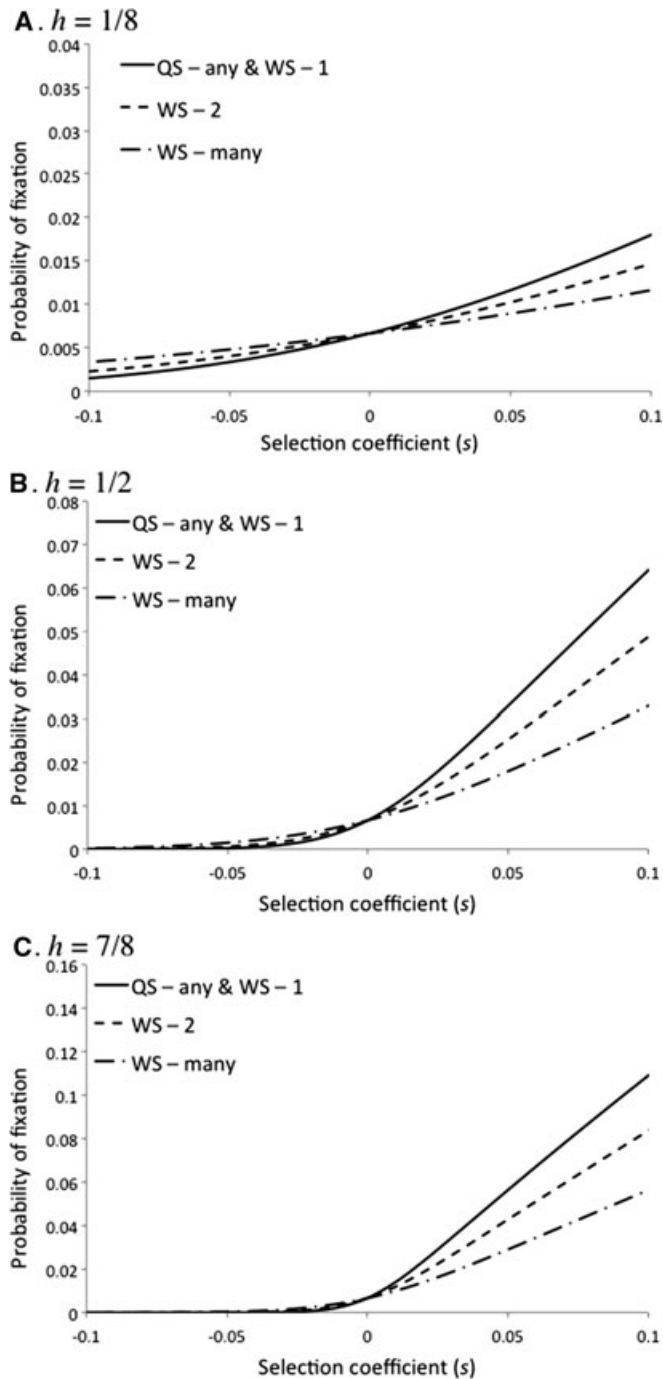
Figure 1. The probability of fixation of a newly arising allele in a haplodiploid population of size $N = 100$. Panels A, B, and C are for the dominance coefficient of the new allele, h, equal to 1/8, 1/2, and 7/8, respectively. The initial frequency of the new allele is 1/150. QS–any: queen selection, with any number of mates. WS–1: worker selection, singly mated queen. WS–2: worker selection, doubly mated queen. WS–many: worker selection, multiply mated queen. Note the twofold change in scale on the y-axis for each successive panel.
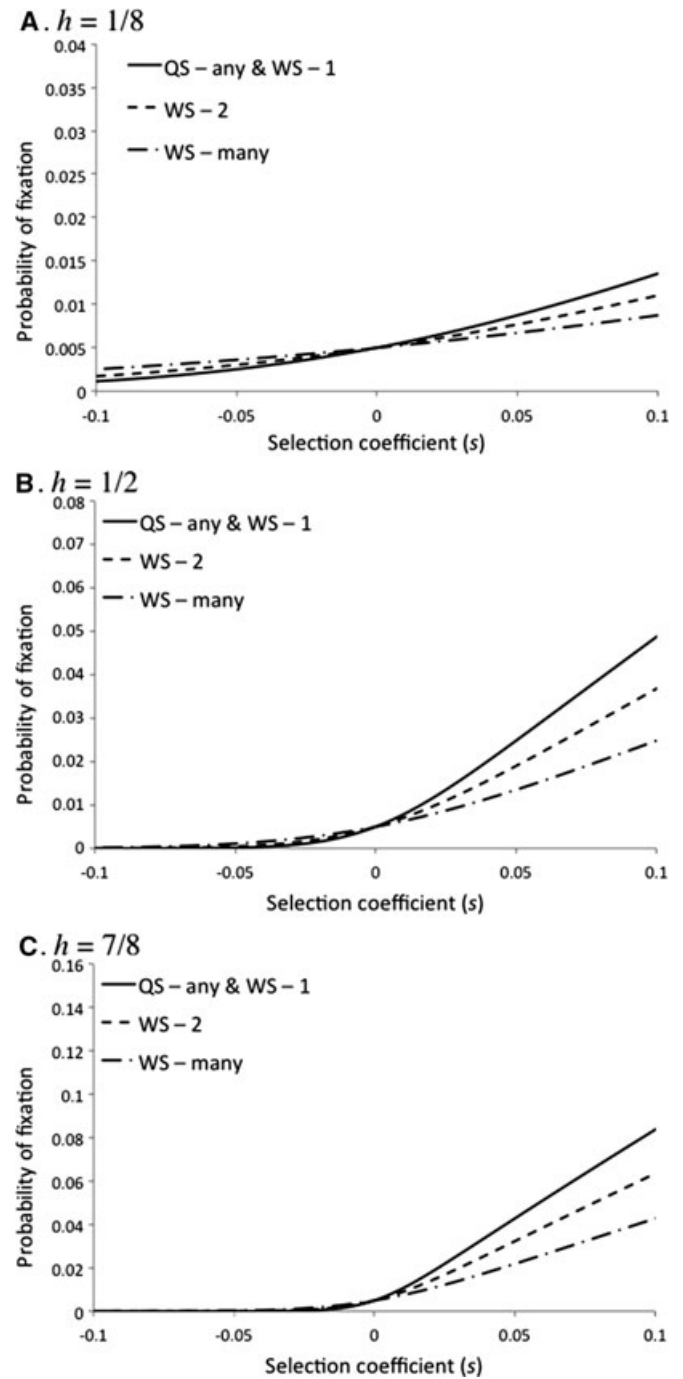
Figure 2. The probability of fixation of a newly arising allele in a diploid population of size $N = 100$. Panels A, B, and C are for the dominance coefficient of the new allele, h, equal to 1/8, 1/2, and 7/8, respectively. The initial frequency of the new allele is 1/200. QS–any: queen selection, with any number of mates. WS–1: worker selection, singly mated queen. WS–2: worker selection, doubly mated queen. WS–many: worker selection, multiply mated queen. Note the twofold change in scale on the y-axis for each successive panel.

Table 3. Relatedness (b_{Ex}) between the individual(s) (*E*) expressing the locus that affects fitness (queen or workers) to the reproductives (*x*) heading the colony (queen and males). b_{EQ} is the relatedness of the expresser to the queen and b_{EM} is the relatedness of the expresser to the male(s) mated to the queen. The last column represents the weighted averages of the two relatedness measures ($\bar{b}$): the weights are both equal to $^1/_2$ for diploids and are equal to $^2/_3$ and $^1/_3$ for the queen and male(s), respectively, in haplodiploids, reflecting their relative genetic contribution to the reproductives of the next generation when the sex ratio is even.

	Expressor (*E*)	Queen matings	b_{EQ}	b_{EM}	$\bar{b}$
Haplodiploid	Queen	Any	1	0	$^2/_3$
	Worker	1	$^1/_2$	1	$^2/_3$
	Worker	2	$^1/_2$	$^1/_2$	$^1/_2$
	Worker	Many	$^1/_2$	0	$^1/_3$
Diploid	Queen	Any	1	0	$^1/_2$
	Worker	1	$^1/_2$	$^1/_2$	$^1/_2$
	Worker	2	$^1/_2$	$^1/_4$	$^3/_8$
	Worker	Many	$^1/_2$	0	$^1/_4$

rate per generation (last column of Table 2A and B) is simply the product of the number of new adaptive mutations that arise per generation ($= 2N\mu$ in diploids, $3/2N\mu$ in haplodiploids) and $2\bar{b}hs$, where $\bar{b}$ is the average of the relatedness for the expressor to the queen and males weighted by their respective genetic contribution to the reproductives produced by the colony (Table 3).

Discussion

We have presented the results of simple, one-locus models to determine the rate of evolution of genes with direct and indirect selective effects in social insects. As demonstrated by Linksvayer and Wade (2009), we have shown that as the number of queen matings increases, the efficacy of worker selection declines and the rate of adaptive evolution declines. For the same reason, less effective selection, mildly deleterious alleles are expected to fix at a higher rate under worker selection than queen selection when the queen mates multiply. These effects arise because multiple mating reduces the relatedness of workers to the queen's mates, which decreases the efficacy of worker selection. Whether the overall effect is that loci that are subject to queen selection will evolve faster than loci subject to worker selection will depend on the fitness distribution of newly arising alleles and the relative population size. As the population size gets large, selection will dominate the effects of drift, and the probability of substitution of (mildly) deleterious alleles will decline toward zero. In this case, queen-selected loci will evolve more rapidly under multiple mating because only beneficial and neutral alleles will contribute to substitutions. On the other hand, in small populations, worker-selected loci may evolve more rapidly due to the increased rate of fixation of mildly deleterious alleles.

In species in which queens are singly mated, queen and worker selection are equivalent in terms of rates of substitution. In social species with singly mated queens, queen- and worker-selected loci are thus expected to evolve at the same rate, regardless of population size. This result is in contrast to the primary conclusion of Linksvayer and Wade (2009), who noted that selection is less efficient for loci with indirect social effects regardless of queen mate number. The difference between our results and theirs is caused by our assumption of sex-limited selection. For a rare allele that affects fitness, direct selection acting only on queens results in fewer colonies experiencing selection than if direct selection acted on both parental genotypes, which reduces the efficacy of selection (e.g., in diploids with queen-limited direct selection, a colony with a *Aa* queen mated with a *AA* male experiences direct selection, whereas a colony with a *AA* queen mated to a *Aa* male does not).

The same effect is not seen for worker selection in diploids because, although sons are absent, daughters have an identical genotypic distribution to sons, implying that sex-specific selection when all offspring are female is equivalent to sib-selection when half the offspring are male and half female. Thus, in our model sex-specific selection reduces the effectiveness of direct selection on queens but has no impact on indirect selection through workers. In haplodiploids, we expect there to be a difference between sib-selection and sex-specific sib-selection because the genotypic distributions differ in sons and daughters. However, we do not find this difference between our model and the one analyzed by Linksvayer and Wade because they implicitly assumed female-expressed sib-effects in their haplodiploid model. Thus, compared to Linksvayer and Wade (2009), the efficacy of direct selection in our model is reduced, but the efficacy of indirect selection is the same, which gives identical rates of evolution under queen and worker selection when queens are singly-mated.

We have also shown that, all else being equal, the rate of evolution at loci under queen or worker selection is not affected by their genetic system, diploid versus haplodiploid. Thus, queen-selected loci in diploids are expected to evolve at the same rate as queen-selected loci in haplodiploids. This result is in agreement with previous work on the rate of evolution of autosomal versus X-linked loci. X-linked loci show identical inheritance as haplodiploid loci: males are haploid (XY or XO) and contribute alleles to daughters only, whereas females (XX) are diploid and contribute equally to both sexes. When selection is female-restricted, autosomal and X-linked loci evolve at identical rates, regardless of dominance (Charlesworth et al. 1987; Kirkpatrick and Hall

2004). This effect arises because females are equivalent genetically in diploids and haplodiploids. A difference in the genetic system does affect both the rate of mutation ($2N\mu$ in diploids vs. $3/2N\mu$ in haplodiploids) and the initial frequency of a new allele ($1/(2N)$ in diploids versus $2/(3N)$ in haplodiploids), but these differences exactly compensate for one another. However, this result is not expected to hold in situations in which expression of the fitness locus is not sex limited, for example, in species with workers composed of individuals of both sexes, as may occur in some termites (Watson and Gay 1991).

We found that relatedness measures perfectly predict the eigenvalues associated with the introduction of a new allele. This result is reassuring given the enormous body of work demonstrating the importance of relatedness in predicting the evolution of helping and altruistic behaviors (Bourke and Franks 1995; Crozier and Pamilo 1996; Pamilo et al. 1997; Frank 1998; West et al. 2007; Abbot et al. 2011; Gardner et al. 2011). Examination of Table 2 reveals that selection is effectively stronger in haplodiploids ($|\lambda-1|$ is always larger) than in diploids, which gives a higher probability of fixation for adaptive alleles, and lower probability for deleterious alleles (Fig. 1). This is true even under queen selection, where relatedness coefficients are identical in haplodiploids and diploids. The reason is because queens have higher genetic contribution in haplodiploids ($2/3$ vs. $1/2$) when the sex ratio is even. Even though selection is effectively stronger, haplodiploids show identical rates of substitution to diploids because fewer new mutations enter the population per generation, exactly offsetting the difference.

We utilized relatedness coefficients between expressers and queens and their mates. We could also have chosen to examine the relatedness between expressers and the reproductives produced by the colony. This would imply that the queen-expressed alleles acted on fitness through indirect maternal-effects and the worker-expressed alleles acted through indirect sib-effects. Our conclusions would not change: relatedness weighted by genetic contribution (Table 3) would still have predicted the eigenvalues (analysis not shown). However, our study was motivated by an attempt to understand molecular evolution data on queen- and worker-expressed genes, and, sex-limited expression is usually considered to imply sex-limited direct selection (Parisi et al. 2004; Ellegren and Parsch 2007; Mank et al. 2008). Further, we consider differences in colony fitness to be due to the number of reproductives produced, which is a direct fitness effect of a female and her mate(s) rather than an indirect maternal effect.

Queller (1992a, b) has shown that the change in the breeding value for an altruistic trait in a population can be written in terms of selection differentials for costs and benefits of altruism and heritabilities for the cost in the actor and for the benefit in the recipient. These heritabilities are tightly connected to relatedness, because both heritability and relatedness measure genetic similarities between group members. Because relatedness predicts the eigenvalue in our model, this suggests that the reduction in the eigenvalue with more queen mating is caused by a reduction in the transmission/heritability of the workers' fitness through their parents. In our model, selection acting through the phenotype(s) of workers affects evolution only to the extent that the fitness alleles are transmitted through the queen and her mates to the next generation.

The examination of relatedness coefficients provides insight into the expectations for scenarios not explicitly considered here. For example, in a diploid species having both male and female workers, as may be the case in some termites (Muller and Korb 2008), the rate of evolution under worker selection would be the same as calculated with sex-limited selection, because relatedness is not different for males versus female workers to the queen and her mate(s). In a species with multiple queens, as seen in several species and taken to the extreme in unicolonial ants (Helantera et al. 2009), relatedness for workers to the queens and their mates is likely close to zero, making worker-expressed loci evolve essentially neutrally because adaptive evolution and purging of deleterious mutations for worker-expressed genes would no longer be possible. Such species are expected to be prone to a steady reduction in worker fitness due to the accumulation of deleterious alleles in the absence of selection.

The dominance of newly arising mutations has a substantial effect on the probability of fixation and rate of evolution (Table 2 and Fig. 1). Recessive deleterious alleles are more likely to fix and recessive adaptive alleles are less likely to fix than dominant alleles. However, because all selection occurs in females, newly arising alleles are under selection in only heterozygotes to a first-order approximation. For this reason, the dominance coefficient cancels out when calculating the relative rates of evolution. Dominance is expected to affect relative rates of evolution if selection occurs in haploid males as for sib-effects in colonies containing sons or with direct selection in males.

We developed our theoretical framework specifically with the intent of applying it to empirically obtained data in social insects. Testing the theory in hymenopteran social insects first requires the identification of genes that specifically, and distinctly, affect the function of queens and workers, because these will likely show caste-specific selection. Differentially expressed genes between queens and workers represent strong candidates for such loci. Genes expressed at high levels in a particular tissue/developmental stage/sex are often functionally related to its specific phenotype (Dewitt and Scheiner 2004; Pal et al. 2006; Moczek 2010; Snell-Rood et al. 2010; Van Dyken and Wade 2010; Connallon and Clark 2011; Sackton et al. 2011). For example, genes with sex-specific effects are often differentially expressed between sexes (Jin et al. 2001; Ranz et al. 2003; Parisi et al. 2004; Ellegren and Parsch 2007; Mank et al. 2008; Jiang and Machado 2009). Thus,
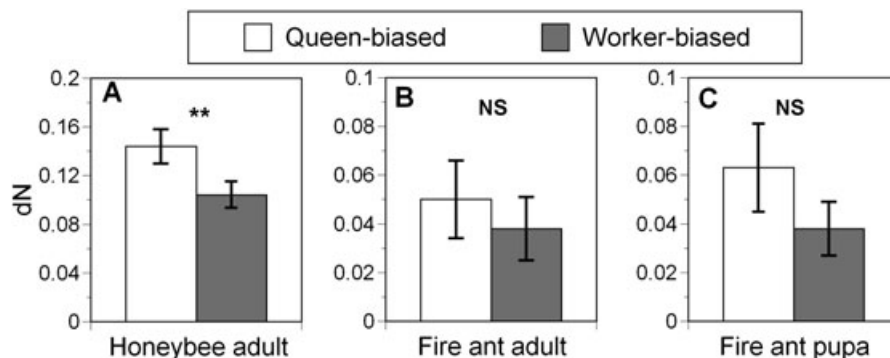
Figure 3. Rates of evolution of genes differentially expressed between hymenopteran social insect castes. (A) Queen-biased genes in adult *Apis mellifera* honeybees evolve significantly more rapidly than worker-biased genes (**$P < 0.01$). (B and C) However, queen-biased genes in adult or pupal *Solenopsis invicta* fire ants do not evolve significantly more rapidly than worker-biased genes (NS = not significant). Error bars denote 95% confidence intervals. Data from Hunt et al. (2010) and (2011).

genes with higher expression in reproductive or nonreproductive castes are those most likely to experience direct or kin selection, respectively (Johnson and Linksvayer 2010; Hunt et al. 2011). An increasing number of studies are using genomic techniques to identify genes differentially expressed between castes (reviewed by Goodisman et al. 2008; Smith et al. 2008) and the large numbers of insect genomes being sequenced (Robinson et al. 2011) provides information that can be used to accurately estimate the rates of evolution of genes that are differentially expressed between castes. Thus, contemporary data provide avenues for testing kin selection theory on a genome-wide scale.

We identified two studies that determined the rates of molecular evolution of genes differentially expressed between hymenopteran castes that were appropriate for testing our theory. Remarkably, available data are consistent with the predictions. First, Hunt et al. (2010) examined the rates of molecular evolution in the honeybee, *Apis mellifera. Apis mellifera* represents an interesting taxon to examine rates of gene evolution because *Apis* queens are very promiscuous. Indeed, *A. mellifera* queens always mate with multiple males and frequently mate 10 or more times (Tarpy et al. 2004). Hunt et al. (2010) determined if genes more highly expressed in queens than workers (hereafter queen-biased genes) and more highly expressed in workers than queens (worker-biased genes) evolved at different rates. Our model predicts that queen- and worker-biased genes in *A. mellifera* should evolve at different rates, assuming that these genes affect queen and worker function, respectively. That is, worker-biased genes would evolve more slowly than queen-biased genes if beneficial alleles had frequently fixed in ancestral populations, whereas the opposite would be true with the fixation of deleterious alleles. Hunt et al. (2010) did, in fact, find that queen- and worker-biased genes evolved at significantly different rates (worker-biased genes evolved more slowly than queen-biased genes; Fig. 3A). These data are consistent with predictions from the model that selection has differentially influenced the rate of

molecular evolution of *A. mellifera* queen- and worker-biased genes.

In a separate study, Hunt et al. (2011) compared the rates of evolution of adult and pupal queen- and worker-biased genes in the fire ant *Solenopsis invicta* (Ometto et al. 2011). Unlike *A. mellifera* queens, *S. invicta* queens typically mate with only a single male (Ross and Fletcher 1985). Consequently, our model predicts that rates of evolution of queen- and worker-biased genes should be similar. Hunt et al. (2011) did, in fact, find that the rates of evolution of queen- and worker-biased genes in *S. invicta* did not differ significantly (Fig. 3B and C). Thus, overall, available data on rates of evolution of caste-biased genes are consistent with expectations from our model; caste-biased genes evolve at significantly different rates in *A. mellifera*, where queens necessarily mate with many males, and not-significantly different rates in *S. invicta*, where queens mate singly.

The meshing of theory with data is striking. However, these results must be viewed with caution for several reasons. First, data are currently available from only two taxa, thereby providing limited power to detect trends. Second, the types of selection pressures operating in queens and workers likely differ, making it unclear if the distribution of selection coefficients for new mutations in the two castes would be similar (Oster and Wilson 1978; Gadagkar 1997). Third, it is not known what fraction of genes differentially expressed between castes would necessarily be subject to caste-specific selection, as modeled in this study (Van Dyken and Wade 2010). Fourth, *S. invicta* and *A. mellifera* show differences in life histories besides the number of mates per queen that may affect the way selection operates in queens versus workers in these taxa (Winston 1987; Tschinkel 2006). Fifth, rates of molecular evolution are affected by factors besides the effects of direct and kin selection as described here (Pal et al. 2006). For example, gene flow between single- and multiple-queen populations of *S. invicta* may affect rates of molecular evolution in both social forms (Goodisman et al. 2000). Finally, the expression levels of

these genes were estimated in different subsets of all possible developmental stages and conditions, thereby complicating direct comparison of the results (Arbeitman et al. 2002; Grozinger et al. 2007; Ometto et al. 2011).

Consequently, it is clear that additional data from more social insect taxa are needed to understand how direct and kin selection have affected rates of molecular evolution. Specifically, future empirical studies should focus on understanding the evolution of genes associated with queen and worker function in diverse taxa exhibiting variation in queen mate number. Multiple mating in hymenopteran social insects is rare but has evolved in some wasps, ants, and bees (Strassmann 2001). Controlled analyses from closely related taxa differing in mate number would be likely to yield additional data that would provide strong tests of the theory.

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Appendix
HAPLODIPLOIDS

Queen-selection; one, two, or multiple matings: The recursion equations for queen-selection with single-mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f_{AA}p_m + \frac{1}{2}f_{Aa}(1+hs)p_m \right),$$

$$f'_{Aa} = \frac{1}{T}\left(f_{AA}q_m + \frac{1}{2}f_{Aa}(1+hs) + f_{aa}(1+s)p_m \right),$$

$$f'_{aa} = \frac{1}{T}\left(f_{aa}(1+s)q_m + \frac{1}{2}f_{Aa}(1+hs)q_m \right),$$

$$p'_m = \frac{1}{T}\left(f_{AA} + \frac{1}{2}f_{Aa}(1+hs) \right),$$

$$q'_m = \frac{1}{T}\left(f_{aa}(1+s) + \frac{1}{2}f_{Aa}(1+hs) \right),$$

where $f_{AA}, f_{Aa}, f_{aa}, p_m,$ and q_m are the frequencies of the *AA*, *Aa*, and *aa* genotypes in queens and the *A* and *a* genotypes in males, respectively. T is a normalizer that ensures that we obtain queen and male frequencies in the next generation and is given by:

$$T = f_{AA} + f_{Aa}(1+hs) + f_{aa}(1+s).$$

The number of mates has no effect on selection acting on the queen or on the average offspring production of a particular queen genotype, so the recursions are the same regardless of the number of queen matings.

Worker-selection; one mating: The recursion equations for queen-selection with single-mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f_{AA}p_m + \frac{1}{2}f_{Aa}\left(1+\frac{1}{2}hs\right)p_m \right),$$

$$f'_{Aa} = \frac{1}{T}\Bigg(f_{AA}q_m(1+hs) \\ + \frac{1}{2}f_{Aa}\left(p_m\left(1+\frac{1}{2}hs\right) + q_m\left(1+\frac{1}{2}hs+\frac{1}{2}s\right) \right) \\ + f_{aa}(1+hs)p_m \Bigg),$$

$$f'_{aa} = \frac{1}{T}\left(f_{aa}q_m(1+s) + \frac{1}{2}f_{Aa}q_m\left(1+\frac{1}{2}hs+\frac{1}{2}s\right) \right),$$

$$p'_m = \frac{1}{T}\left(f_{AA}(1+q_mhs) + \frac{1}{2}f_{Aa}\left(1+\frac{1}{2}hs+q_m\frac{1}{2}s\right) \right),$$

$$q'_m = \frac{1}{T}\left(f_{aa}(1 + p_m hs + q_m s) + \frac{1}{2}f_{Aa}\left(1 + \frac{1}{2}hs + q_m\frac{1}{2}s\right)\right),$$

where

$$T = f_{AA}(1 + q_m hs) + f_{Aa}\left(1 + \frac{1}{2}hs + q_m\frac{1}{2}s\right)$$
$$+ f_{aa}(1 + p_m hs + q_m s).$$

Worker-selection; two matings: The recursion equations for queen-selection with doubly mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f_{AA}\left(p_m^2 + p_m q_m\left(1 + \frac{1}{2}hs\right)\right)\right.$$
$$+ \frac{1}{2}f_{Aa}\left(p_m^2\left(1 + \frac{1}{2}hs\right)\right.$$
$$\left.\left.+ p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)\right)\right),$$

$$f'_{Aa} = \frac{1}{T}\left(f_{AA}\left(p_m q_m(1 + \frac{1}{2}hs) + q_m^2(1 + hs)\right)\right.$$
$$+ f_{aa}\left(p_m^2(1 + hs) + p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)$$
$$+ \frac{1}{2}f_{Aa}\left(p_m^2\left(1 + \frac{1}{2}hs\right)\right.$$
$$+ 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)$$
$$\left.\left.+ q_m^2\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)\right),$$

$$f'_{aa} = \frac{1}{T}\left(f_{aa}\left(q_m^2(1 + s) + p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)\right.$$
$$+ \frac{1}{2}f_{Aa}\left(p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)\right.$$
$$\left.\left.+ q_m^2\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)\right),$$

$$p'_m = \frac{1}{T}\left(f_{AA}\left(p_m^2 + 2p_m q_m\left(1 + \frac{1}{2}hs\right) + q_m^2(1 + hs)\right)\right.$$
$$+ \frac{1}{2}f_{Aa}\left(p_m^2\left(1 + \frac{1}{2}hs\right)\right.$$
$$+ 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)$$
$$\left.\left.+ q_m^2\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)\right),$$

$$q'_m = \frac{1}{T}\left(f_{aa}\left(p_m^2(1 + hs) + 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right.\right.$$
$$\left.+ q_m^2(1 + s)\right)$$
$$+ \frac{1}{2}f_{Aa}\left(p_m^2\left(1 + \frac{1}{2}hs\right) + 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)\right.$$
$$\left.\left.+ q_m^2\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)\right),$$

where

$$T = \left(f_{AA}\left(p_m^2 + 2p_m q_m\left(1 + \frac{1}{2}hs\right) + q_m^2(1 + hs)\right)\right.$$
$$+ f_{Aa}\left(p_m^2\left(1 + \frac{1}{2}hs\right) + 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{4}s\right)\right.$$
$$\left.+ q_m^2\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right)$$
$$+ f_{aa}\left(p_m^2(1 + hs) + 2p_m q_m\left(1 + \frac{1}{2}hs + \frac{1}{2}s\right)\right.$$
$$\left.\left.+ q_m^2(1 + s)\right)\right).$$

Worker-selection; multiple matings: The recursion equations for queen-selection with multiply mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f_{AA}p_m(1 + q_m hs) + \frac{1}{2}f_{Aa}p_m\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)\right),$$

$$f'_{Aa} = \frac{1}{T}\left(f_{AA}q_m(1 + q_m hs) + \frac{1}{2}f_{Aa}\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)\right.$$
$$\left.+ f_{aa}p_m(1 + p_m hs + q_m s)\right),$$

$$f'_{aa} = \frac{1}{T}\left(f_{aa}q_m(1 + p_m hs + q_m s)\right.$$
$$\left.+ \frac{1}{2}f_{Aa}q_m\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)\right),$$

$$p'_m = \frac{1}{T}\left(f_{AA}(1 + q_m hs) + \frac{1}{2}f_{Aa}\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)\right),$$

$$q'_m = \frac{1}{T}\left(f_{aa}(1 + p_m hs + q_m s) + \frac{1}{2}f_{Aa}\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)\right),$$

where

$$T = f_{AA}(1 + q_m hs) + f_{Aa}\left(1 + \frac{1}{2}hs + \frac{1}{2}q_m s\right)$$
$$+ f_{aa}(1 + p_m hs + q_m s).$$

Note that we assume that each queen mates enough times so that the sperm she carries accurately reflects the frequency of males in the population.

DIPLOIDS

Queen-selection; one, two, or multiple matings: The recursion equations for queen-selection with single-mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f^2_{AA} + \frac{1}{2}f_{AA}f_{Aa} + \frac{1}{2}f_{Aa}f_{AA}(1+hs) + \frac{1}{4}f^2_{Aa}(1+hs)\right),$$

$$f'_{Aa} = \frac{1}{T}\left(f_{AA}f_{aa} + \frac{1}{2}f_{AA}f_{Aa} + \frac{1}{2}f_{Aa}(1+hs) + \frac{1}{2}f_{aa}f_{Aa}(1+s) + f_{aa}f_{AA}(1+s)\right),$$

$$f'_{aa} = \frac{1}{T}\left(f^2_{aa}(1+s) + \frac{1}{2}f_{aa}f_{Aa}(1+s) + \frac{1}{2}f_{Aa}f_{aa}(1+hs) + \frac{1}{4}f^2_{Aa}(1+hs)\right),$$

where f_{AA}, f_{Aa}, f_{aa}, are the frequencies of the *AA*, *Aa*, and *aa* genotypes in reproductives, respectively. T is a normalizer that ensures that we obtain frequencies in the next generation and is given by:

$$T = f_{AA} + f_{Aa}(1+hs) + f_{aa}(1+s).$$

The number of mates has no effect on selection acting on the queen or on the average offspring production of a particular queen genotype, so the recursions are the same regardless of the number of queen matings.

Worker-selection; one mating: The recursion equations for queen-selection with single-mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f^2_{AA} + f_{AA}f_{Aa}\left(1+\frac{1}{2}hs\right) + \frac{1}{4}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)\right),$$

$$f'_{Aa} = \frac{1}{T}\left(2f_{AA}f_{aa}(1+hs) + f_{AA}f_{Aa}\left(1+\frac{1}{2}hs\right) + \frac{1}{2}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right) + f_{Aa}f_{aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right)\right),$$

$$f'_{aa} = \frac{1}{T}\left(f^2_{aa}(1+s) + f_{aa}f_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right) + \frac{1}{4}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)\right),$$

where

$$T = f^2_{AA} + 2f_{AA}f_{Aa}\left(1+\frac{1}{2}hs\right) + 2f_{AA}f_{aa}(1+hs) + f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right) + 2f_{Aa}f_{aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right) + f^2_{aa}(1+s).$$

Worker-selection; two matings: The recursion equations for queen-selection with doubly mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\left(f_{AA}\left(f^2_{AA} + \frac{3}{2}f_{AA}f_{Aa}\left(1+\frac{1}{4}hs\right)\right.\right.$$
$$+ f_{AA}f_{aa}\left(1+\frac{1}{2}hs\right)$$
$$+ \frac{1}{2}f^2_{Aa}\left(1+\frac{1}{2}hs\right) + \frac{1}{2}f_{Aa}f_{aa}\left(1+\frac{3}{4}hs\right)\Big)$$
$$+ f_{Aa}\left(\frac{1}{2}f^2_{AA}\left(1+\frac{1}{2}hs\right)\right.$$
$$+ \frac{3}{4}f_{AA}f_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{8}s\right)$$
$$+ \frac{1}{2}f_{AA}f_{aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)$$
$$+ \frac{1}{4}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)$$
$$+ \frac{1}{4}f_{Aa}f_{aa}\left(1+\frac{1}{2}hs+\frac{3}{8}s\right)\Big)\Big),$$

$$f'_{Aa} = \frac{1}{T}\left(f_{AA}\left(\frac{1}{2}f_{AA}f_{Aa}\left(1+\frac{1}{4}hs\right)\right.\right.$$
$$+ f_{AA}f_{aa}\left(1+\frac{1}{2}hs\right) + \frac{1}{2}f^2_{Aa}\left(1+\frac{1}{2}hs\right)$$
$$+ \frac{3}{2}f_{Aa}f_{aa}\left(1+\frac{3}{4}hs\right) + f^2_{aa}(1+hs)\Big)$$
$$+ f_{Aa}\left(\frac{1}{2}f^2_{AA}\left(1+\frac{1}{2}hs\right)\right.$$
$$+ f_{AA}f_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{8}s\right)$$
$$+ f_{AA}f_{aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)$$
$$+ \frac{1}{2}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{4}s\right)$$
$$+ f_{Aa}f_{aa}\left(1+\frac{1}{2}hs+\frac{3}{8}s\right)$$
$$+ \frac{1}{2}f^2_{aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right)\Big)$$
$$+ f_{aa}\left(f^2_{AA}(1+hs)\right.$$
$$+ \frac{3}{2}f_{AA}f_{Aa}\left(1+\frac{3}{4}hs+\frac{1}{4}s\right)$$
$$+ f_{AA}f_{aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right)$$
$$+ \frac{1}{2}f^2_{Aa}\left(1+\frac{1}{2}hs+\frac{1}{2}s\right)$$
$$+ \frac{1}{2}f_{Aa}f_{aa}\left(1+\frac{1}{4}hs+\frac{3}{4}s\right)\Big)\Big),$$

$$f'_{aa} = \frac{1}{T}\Bigg(f_{Aa}\Big(\frac{1}{4}f_{AA}f_{Aa}\Big(1+\frac{1}{2}hs+\frac{1}{8}s\Big)$$

$$+\frac{1}{2}f_{AA}f_{aa}\Big(1+\frac{1}{2}hs+\frac{1}{4}s\Big)$$

$$+\frac{1}{4}f_{Aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{4}s\Big)$$

$$+\frac{3}{4}f_{Aa}f_{aa}\Big(1+\frac{1}{2}hs+\frac{3}{8}s\Big)$$

$$+\frac{1}{2}f_{aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)\Big)$$

$$+f_{aa}\Big(\frac{1}{2}f_{AA}f_{Aa}\Big(1+\frac{3}{4}hs+\frac{1}{4}s\Big)$$

$$+f_{AA}f_{aa}\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)$$

$$+\frac{1}{2}f_{Aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)$$

$$+\frac{3}{2}f_{Aa}f_{aa}\Big(1+\frac{1}{4}hs+\frac{3}{4}s\Big)$$

$$+f_{aa}^2(1+s)\Big)\Bigg),$$

where

$$T = f_{AA}\Big(f_{AA}^2 + 2f_{AA}f_{Aa}\Big(1+\frac{1}{4}hs\Big)$$

$$+2f_{AA}f_{aa}\Big(1+\frac{1}{2}hs\Big)+f_{Aa}^2\Big(1+\frac{1}{2}hs\Big)$$

$$+2f_{Aa}f_{aa}\Big(1+\frac{3}{4}hs\Big)+f_{aa}^2(1+hs)\Big)$$

$$+f_{Aa}\Big(f_{AA}^2\Big(1+\frac{1}{2}hs\Big)+2f_{AA}f_{Aa}\Big(1+\frac{1}{2}hs+\frac{1}{8}s\Big)$$

$$+2f_{AA}f_{aa}\Big(1+\frac{1}{2}hs+\frac{1}{4}s\Big)$$

$$+f_{Aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{4}s\Big)$$

$$+2f_{Aa}f_{aa}\Big(1+\frac{1}{2}hs+\frac{3}{8}s\Big)$$

$$+f_{aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)\Big)$$

$$+f_{aa}\Big(f_{AA}^2(1+hs)+2f_{AA}f_{Aa}\Big(1+\frac{3}{4}hs+\frac{1}{4}s\Big)$$

$$+2f_{AA}f_{aa}\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)$$

$$+f_{Aa}^2\Big(1+\frac{1}{2}hs+\frac{1}{2}s\Big)$$

$$+2f_{Aa}f_{aa}\Big(1+\frac{1}{4}hs+\frac{3}{4}s\Big)+f_{aa}^2(1+s)\Big).$$

Worker-selection; multiple matings: The recursion equations for queen-selection with multiply mated queens are as follows:

$$f'_{AA} = \frac{1}{T}\Bigg(f_{AA}\Big(f_{AA}+\frac{1}{2}f_{Aa}\Big)\Big(1+\Big(f_{aa}+\frac{1}{2}f_{Aa}\Big)hs\Big)$$

$$+\frac{1}{2}f_{Aa}\Big(f_{AA}+\frac{1}{2}f_{Aa}\Big)\frac{1}{2}\Big(2+hs+\Big(f_{aa}+\frac{1}{2}f_{Aa}\Big)s\Big)\Bigg),$$

$$f'_{Aa} = \frac{1}{T}\Bigg(f_{AA}(f_{aa}+\frac{1}{2}f_{Aa})\Big(1+(f_{aa}+\frac{1}{2}f_{Aa})hs\Big)$$

$$+\frac{1}{2}f_{Aa}\frac{1}{2}\Big(2+hs+(f_{aa}+\frac{1}{2}f_{Aa})s\Big)$$

$$+f_{aa}(f_{AA}+\frac{1}{2}f_{Aa})\Big(1+(f_{AA}+\frac{1}{2}f_{Aa})hs$$

$$+(f_{aa}+\frac{1}{2}f_{Aa})s\Big)\Bigg),$$

$$f'_{aa} = \frac{1}{T}\Bigg(f_{aa}\Big(f_{aa}+\frac{1}{2}f_{Aa}\Big)\Big(1+\Big(f_{AA}+\frac{1}{2}f_{Aa}\Big)hs$$

$$+\Big(f_{aa}+\frac{1}{2}f_{Aa}\Big)s\Big)$$

$$+\frac{1}{2}f_{Aa}(f_{aa}+\frac{1}{2}f_{Aa})\frac{1}{2}\Big(2+hs+(f_{aa}+\frac{1}{2}f_{Aa})s\Big)\Bigg),$$

where

$$T = f_{AA}\Big(1+(f_{aa}+\frac{1}{2}f_{Aa})hs\Big)$$

$$+f_{Aa}\frac{1}{2}\Big(2+hs+(f_{aa}+\frac{1}{2}f_{Aa})s\Big)$$

$$+f_{aa}\Big(1+(f_{AA}+\frac{1}{2}f_{Aa})hs+(f_{aa}+\frac{1}{2}f_{Aa})s\Big).$$

Note that we assume that each queen mates enough times so that the sperm she carries accurately reflects the frequency of males in the population.