

Genetics of Vestigial Wing in Honey Bees (*Apis mellifera* L.) and Maintenance of the Recessive Alleles by Natural Selection in the Haplo-Diploid System

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ABSTRACT

The vestigial winged drones in a honey bee (*Apis mellifera* L.) colony was observed. The vestigial winged drone cannot fly. The genetic study showed that the trait is controlled by a recessive allele.

Maintenance of the recessive allele by natural selection was examined. The recessive lethal allele can be rapidly eliminated in the haplo-diploid system by natural selection, unless the selective advantage of heterozygote over the wild type homozygote is greater than 1.0. The allele frequency theoretically can never be greater than 0.50 no matter how large the selective advantage of the heterozygote. Based on the average weight gain of the colonies, no appreciable heterosis was demonstrated.

The honey bee (*Apis mellifera* L.) is a social insect. In a colony of honey bees, there are the queen, a large number of workers, and a certain number of drones. The number of workers and drones present varies among colonies, and is dependent upon many factors. The queen and worker honey bees are females and are diploid; the drones are males, which are developed from unfertilized eggs. Thus the drones are haploid, and the drone progenies give gametic ratios of their heterozygous mothers. The fertilized egg can develop into either a queen or a worker dependent upon the quantity and quality of food they receive at the larval stage. If a larva hatched from a fertilized egg feeds on abundant royal jelly through its larval stage it will develop into a queen; on the other hand if the larva receives royal jelly for only the first three days followed by honey and pollen it will become a worker. The females in the honey bee colony are divided

into two castes, the queens and the workers.

The queens and workers are highly dimorphic and display very different behaviors and physiologies. A queen normally lives from two to four years, and can produce thousands of female and male offspring. Workers live a much shorter time (about 6 weeks in the peak season and longer in the winter), but display many highly sophisticated behaviors. In the colony, they build nests, defend the hive, clean the brood nest, nurse the brood, gather food, feed and groom the queen, and maintain the optimum temperatures of the hive in both winter and summer.

A drone is a highly specialized animal. His genitalia, proportional to his body size, are larger than those of almost all other animals (Morse 1975). His sole function is to mate, but he mates only once. In contrast, queen honey bees mate several times, and they mate in flight. After mating the queens stay inside

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the hives all the time unless swarming.

When the queen honey bee in a colony becomes weak and old, or dies suddenly, the workers will raise a new queen. If the colony is very populous when the food source is abundant, the workers may raise from several to over a dozen queen cells. Before the new queens emerge from the queen cells, the queen with about 50-60% of the workers swarm out and establish a colony in a new place. So swarming is a part of honey bees life cycle and is the way of increasing their population. One of the virgin queens emerged from the queen cells in the old nest will mate and become the queen of the old hive.

Although the history of beekeeping is very old, honey bees are not domesticated. It is difficult to study genetics of honey bees and to breed them because they mate in the air, and control mating was impossible until the technique of artificial insemination was developed. Since honey bees have the haplo-diploid chromosome number in two sexes, many geneticists are interested in studying their genetics. However, progress has been slow (Rothenblyler *et al.* 1969). The males are haploid so that recessive mutations will express phenotypically in the males. Many such recessive mutant eye colors were studied (Kerr and Laidlow 1956, Laidlow *et al.* 1954a, 1965b, Laidlow *et al.* 1953). The evolution of social bees, sex determination and genetic variation and behaviors of different races of *Apis mellifera* L. have been reported (Kerr 1969, 1974, 1976).

In addition to several eye color mutants, several mutations which affect the wing size and shape of honey bees were reported. Rudimentary wing in the honey bee was first reported by Hachinohe and Ohishi (1953). The rudimentary wing is a dominant phenotypic trait. The wrinkled, short and truncate wing are recessive traits and each of them is controlled by

a single locus (Kerr and Laidlow 1956, Laidlow *et al.* 1965a, 1965b). The wrinkled wing (wr) showed incomplete penetrance, and expression of this phenotype is variable (Laidlow *et al.* 1965b). Wrinkled wing bees can fly if the abnormality is not extreme, whereas the short-winged (sh) and truncated winged (tr) adult drones cannot fly (Laidlow *et al.* 1965b).

In 1975 a large number of vestigial winged drones were observed in one of the colonies in my apiary at Durham, New Hampshire. The queen was given the name Meilin for identification. The vestigial winged drones appeared to be normal except their wings were rudimentary and deformed. Since they cannot fly, they cannot mate. This paper reports the inheritance of the vestigial winged trait and how this trait can be maintained in the population.

MATERIALS AND METHODS

Since the drones are haploid, and represent the gametic ratio of their heterozygous mother, the number of normal and vestigial winged drones from the hive was counted for two seasons. Eggs from Queen Meilin, which produced vestigial winged drones, were used to raise queens. These queens were used to establish new colonies. The queens were allowed to mate naturally. All these sister colonies were maintained in the same place without any other non-experimental colonies near-by. The initial number of worker bees introduced in each of those experimental colonies was approximately 4,000 and drones were deliberately excluded at the time of establishment. Each colony initially was fed 5 kg of sugar syrup. In order to compare the performances of the colonies the weight of the colonies was taken at the beginning of June and at the end of July. The number of normal and vestigial winged drones was scored from each colony. The experiment was conducted

for two years.

RESULTS AND DISCUSSION

The number of vestigial and normal winged drones does not deviate from a 1:1 ratio (Table 1). Since drones represent the gametic ratio of their heterozygous mother, the results indicate that the vestigial winged trait is caused by a recessive gene. This observation was further supported by the number of Meilin's daughter which produced vestigial winged drones. A total of 25 queens were raised from Queen Meilin's eggs, and 14 of them produced both normal and vestigial winged drones, and 11 produced only normal winged drones. Again the ratio of queens producing vestigial winged drones and those producing only normal winged drones approximates one to one. Based on the results it was concluded that the genotypes of those 14 queens were, like their mother, heterozygous for the vestigial wings. Table 2 shows the number of normal and vestigial winged drones counted in nine colonies which produced vestigial winged drones. In each colony, the number of normal and vestigial winged drones fits a one to one ratio well. The evidence strongly supports that the vestigial wing trait is conditioned by a recessive gene, and those queens that produced vestigial winged drones were heterozygous for the gene. In *Drosophila melanogaster* the vestigial wing is also a recessive trait controlled by a single gene locus (Sinnott *et al.* 1958). Laidlow *et al.* (1965b)

Table 1. Number of normal and vestigial winged honey bee drones in samples produced by Queen Meilin

Year	Normal winged	Vestigial winged	<i>p</i>
1975	76	70	>0.5
1976	58	49	>0.25
Total	134	119	>0.25

Table 2. Number of normal and vestigial honey bee drones produced by the queens descended from Queen Meilin

Queen	Normal winged	Vestigial winged	<i>p</i>
A	28	25	>0.5
C	43	39	>0.5
E	34	32	>0.75
F	49	43	>0.5
H	55	69	>0.1
K	38	30	>0.25
L	74	68	>0.5
M	36	29	>0.25
Total	357	335	>0.25

reported that the wrinkled wing trait showed incomplete penetrance with wild type eye color but it showed complete penetrance in brick-eyes drones. Since the normal winged and vestigial winged drones were almost in equal number from the heterozygous queens, the vestigial wing must be or nearly complete penetrance. It also indicates that the viability of the vestigial winged drones must be equal or nearly equal to the normal winged drones. The viability of the short winged and truncate winged drone is significantly lower than the wild type drone. These two traits are semilethal (Laidlow *et al.* 1965b).

In order to examine whether heterozygous superiority exists in heterozygous queens and worker honey bees the weight increase of the colonies was recorded. The weight gain represents increase in population size and in food storage. The colony which can maintain a large work force and store more food would have greater chance of survival and of producing viable swarms. The average weight gain between the colony with homozygous and heterozygous queens is not significantly different in both years (Table 3). The results show that

Table 3. Average weight gained by colonies headed by wild type homozygous and heterozygous queens

Year	Queens	Number colonies	Weight gained (kg)	S_r^2
1976	Heterozygous	8	15.90	2.81
	Homozygous	5	14.04	5.07
1977	Heterozygous	6	16.6	1.73
	Homozygous	6	16.1	1.96

there is no clear heterozygous superiority. Heterosis in the honey bee (*Apis mellifera* L.) has been reported by Cale and Gowen (1956). They demonstrated that the hybrid queens increased oviposition rate by 35.5% and honey yield by 15% over the inbred queens. Bruckner (1976) reported that inbreeding in honey bees (*Apis mellifera*) significantly reduces developmental homeostasis. The intra individual variability of left vs right wings was larger in drones than in workers, and highly homozygous workers were more variable than normally heterozygous workers. The broodnest temperature in early spring is lower and less stable in inbred colonies than in non-inbred colonies (Brucknes 1978).

In the haplo-diploid system disadvantageous genes should be eliminated faster by natural selection in the haploid individuals (drones) and beneficial genes are likely to increase in frequency faster than in the diploid system. The heterosis in the haplo-diploid system can be effective only in the diploid individuals (females). Since the vestigial winged drones, although viable cannot fly thus cannot mate with queens, its Darwinian fitness is zero. Thus, the wild type drones are the only males in the mating population (Table 4). In the female population the recessive homozygote cannot be produced in the natural condition, thus there are only two genotypes. Let A represent the wild type allele with frequency P and a the

Table 4. The genotypes and relative fitness in the female and male honey bees with respect to the vestigial wing locus

	Female			Male	
	AA	Aa	aa	A	a
genotype	AA	Aa	aa	A	a
frequency	P	q	0	1	0
fitness	1	1+s	0	1	0

vestigial wing allele with frequency q in the female population. The frequency of allele A in the male population is 1. Therefore, the genotypes AA and Aa represent the gametic frequency A and a respectively. Now, let the relative fitness of AA be 1 and Aa be $1+s$ (see Table 4). Since there are only wild type drones available for mating, both wild type and heterozygous queens mate with the wild type drones. Therefore, the wild type queens produce only wild type progeny, however, the heterozygous queens produce one half of wild type and one half of heterozygous females, and one half of the wild type drones and one half of vestigial winged drones. Since there are only wild type drones, the change in phenotypic frequencies by selection will occur in the female population, which we consider as the queen population. Since there is one queen in each colony, we can equate the queen population to the number of colonies. The average population fitness in females will be $\bar{W} = \sum f_i w_i = P + q(1+s)$, where f_i is the frequency of the i th genotype, and w_i the fitness of the i th genotype. One half the Aa progeny is AA , and the other half is Aa . Therefore, after one generation of selection, the Aa genotypic frequency (q_1)

$$q_1 = \frac{\frac{1}{2} q(1+s)}{1+sq} = \frac{q(1+s)}{2(1+sq)} \quad (1)$$

The change in genotypic frequency of Aa , Δq , equals,

$$\begin{aligned} \Delta q = q_1 - q &= \frac{q(1+s)}{2(1+sq)} - q \\ &= \frac{q(s-2sq-1)}{2(1+sq)} \end{aligned} \quad (2)$$

If s is 0, the two genotypes have equal fitness, and $q_1 = \frac{1}{2}q$. Therefore, selection against the recessive lethal on the haploid males will reduce the recessive allele frequency by half in each generation even in the wild type homozygous and heterozygous female which have equal fitness.

When the population reaches equilibrium, $\Delta q = 0$; so equate the formula (2) to 0, $q(s-2sq-1)/2(1+sq) = 0$.

$$q(s-2sq-1) = 0 \quad (3)$$

Solve the equation (3) for the equilibrium allele frequency, \hat{q}

$$\begin{aligned} \hat{q} &= 0, \text{ or} \\ (s-2sq-1) &= 0 \\ \hat{q} &= \frac{-(1-s)}{2s} \end{aligned} \quad (4)$$

$\hat{q} = 0$ is a trivial solution.

Based on (4), various values of s and \hat{q} are presented in Table 5. When $s = 1.0$, that is, the fitness value of the heterozygous female is twice that of the wild type homozygote, the frequency of the recessive allele, $\hat{q} = 0$, i. e. the

Table 5. The equilibrium frequency (\hat{q}) for a recessive lethal in the haplo-diploid system with various values of selective advantage (s) of the heterozygous in females

S	$\hat{q} = -(1-s)/2s$	S	$\hat{q} = -(1-s)/2s$
1.0	0	3.0	0.333
1.1	0.045	4.0	0.375
1.4	0.143	10.0	0.450
1.6	0.188	50.0	0.490
1.8	0.222	100.0	0.495
2.0	0.250	200.0	0.498
2.5	0.300		

recessive lethal allele cannot be maintained in the population. When $s = 3.0$, $\hat{q} = 0.25$, that is when the fitness value of the heterozygote is four times higher than the wild type homozygote, the recessive lethal allele frequency will be 0.25. The limit of theoretically possible highest frequency for the recessive lethal allele is 0.5, that is when AA is also lethal, and the entire population is heterozygote Aa .

It is clear that the recessive lethal allele cannot be easily maintained in haplo-diploid system by the heterosis alone, unless the relative fitness of heterozygote is more than twice greater than that of the wild type homozygote. This theoretical calculation can also apply to the short wing and truncate wing alleles reported by Laidlow *et al.* (1965b). However, the calculation cannot directly apply to the wrinkled allele because of its incomplete penetrance (Laidlow *et al.* 1965b). Some of the wrinkled wing genotype drones can fly and may be able to pass the wrinkled wing allele to the following generation.

By contrast, in a diploid population a recessive lethal allele can be maintained by a small heterozygote advantage (Kiang and Libby) 1972. For example with a heterozygote advantage of 0.1 ($s = 0.1$) a recessive allele can be maintained with the equilibrium frequency of 0.091 in a diploid population.

Kerr examined the equilibrium allele frequencies maintained by heterosis in a haplo-diploid population, and arrived at the conclusion that the equilibrium can be maintained if the frequency P of A and q of a would satisfy the following equation (cited in Kerr and Laidlow 1956).

$$\frac{P}{a} = \frac{W_A + W_a - 2W_a^2}{W_A + W_a - 2W_A^2} \quad (5)$$

where the W_A is relative fitness of the females AA and the males. A , W_a is the average relative

fitness of the females aa and the males a_s and by assuming that the relative fitness of Aa is 1. This equation can be satisfied only if W_A and W_a are smaller than 1, and the difference between W_A and W_a cannot be greater than that permitted by the formula

$$W_A < \frac{1 + \sqrt{1 + 8W_a}}{4}$$

If $W_a = 0$, $W_A < 0.50$, this condition is similar to the condition in formula (4) on this paper. For comparison, \hat{q} is calculated by using formula (5) with various values of W_A and its corresponding value of s (Table 6). The values of \hat{q} in Tables 5 and 6 are almost identical.

Table 6. The equilibrium frequency of a recessive lethal allele (\hat{q}) with various values of W_A with corresponding values of s . (see the text formulae (4) and (5))

s	W_A	\hat{q}
1.0	0.5	0
1.1	0.476	0.046
1.4	0.476	0.142
1.8	0.357	0.222
2.0	0.333	0.250
2.5	0.286	0.299
3.0	0.250	0.333
10.0	0.091	0.448
50.0	0.020	0.487

In natural conditions a queen honey bee (*Apis mellifera* L.) normally lives 2-3 years and the number of established queens produced by each queen is quite limited, on average probably not more than two, because success of a swarm in establishing a colony is slightly smaller than 25% (Kerr 1974). Therefore, genetic drift can play an important role in the gene frequency change in the honey bee population. With the combination of the effect of selection and

genetic drift, a new mutant allele can be lost rapidly from the gene pool.

The heterosis can be demonstrated either in queens or workers or in both. In natural conditions new queens are made by workers when the colony is preparing for swarming or the queen is dying. In preparing for swarming, the colony usually makes from a few to more than a dozen queen cells. The old queen swarms with about 60% of the workers in the colony before the new queens emerge. As soon as new queens emerge, they will search for the queen cells and sting the helpless larvae to death. If two virgin queens meet in the colony, they will fight until one is killed. Thus, each colony has only one queen. There are two traits which assist survival of queens in competition (1) fast development at larval stage, and (2) the aggressive behavior. If a heterozygous queen has one or both traits it will have a better chance to win the virgin queen competition. A colony with a highly productive queen will be very populous. A large colony can gather and store a large quantity of food which is essential for the survival of the colony. Although the causes of swarming are very complex in natural conditions, the larger colony is more likely to swarm than the small colony. The larger colony will have a larger swarm, and the larger swarm will have a better chance to establish a new colony successfully. The longer the life-span, the more productive the queens will be.

The worker honey bees are diploid, therefore, they can manifest heterosis. If the worker bees of certain genotype are aggressive and very diligent, they can gather and store a large amount of food, and maintain a large colony. Also, if the workers of certain genotypes can live longer, they are likely to store more food. The workers displaying these traits can certainly increase the fitness of the colony.

The behavior, developmental rate and life span of the queens and workers heterozygous for vestigial wing were not examined. However, the weight gains of the colonies were recorded. The results showed that there were no differences in average weight gain between the wild type homozygous and heterozygous colonies. Therefore, the clear heterosis of the heterozygous for vestigial wing was not demonstrated. Thus, recessive vestigial wing allele cannot be maintained in the honey bee population. The vestigial wing allele in the Queen Meilin may be a new mutation occurred in one of the gametes which developed into Queen Meilin.

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蜜蜂之隱性短翅對偶基因如何在天擇過程中被保留下來

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摘 要

在蜜蜂 (*Apis mellifera* L.) 之蜂巢中，短期的雄蜂是無法飛翔的，由遺傳學上的實驗發現，短翅的特性是由隱性的基因所控制。

此實驗的目的，即研究這短翅的隱性基因如何在天擇的過程中，被保留下來。通常一對隱性致死的基因，在天擇的過程中，是很快會被淘汰掉，除非是基因結合子 (heterozygote) 之選擇益處 (selective advantage) 要大於 (>1.0) 野生型之同基因結合子 (homozygote)。理論上，在異基因結合子上的基因，不管它有多大的天擇益處 (selective advantage)，它的出現頻率是不會大於 0.5。在觀察各蜂巢的過程中，由蜂巢之平均重量所得，並沒有明顯的顯示出雜交優勢 (heterosis) 的現象。