

POPULATION GENETIC STUDIES IN BEES. 2. SEX-LIMITED GENES¹

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Until 1945, the Hymenoptera were considered to be somewhat limited in evolutionary possibilities, since they could not have the capacity to build a concealed variability, be it of either detrimental or lethal genes. This assumption is denied by the enormous number of species in this order (which is only surpassed by the Coleoptera and Diptera), by the recent demonstration of evolution in the social lives and learning abilities of bees (Kerr 1969, 1974a; Pessotti, 1967; Wilson, 1971; Michener, 1974), and by the theoretical paper by Hartl (1972) who considers that haplo-diploid populations should evolve one-third faster than diplo-diploids.

White (1945) suggested the possibility that sex-limited genes could be of importance in the genetic variability of the Hymenoptera. Kerr (1951) demonstrated that this is the case for certain genes in natural populations of stingless bees (*Meliponini*). Kerr (1951, 1967a) showed one mathematical basis which indicates that the Hymenoptera with sex-limited genes (whose equilibrium or elimination of genes follows the same rules and formulae as in diploid populations) can exploit the advantages of diploid populations (changing environments) and, with the remaining genes, they would exploit the advantages of the haplo-diploid populations (stable environments). The role of female-

limited genes in the variability of 59 X chromosomes was demonstrated by Kerr and Kerr (1952), and confirmed by Drescher (1964) with the study of 234, Gallo (1970) with 192 and Renesto (1973) with 47 X chromosomes. Drescher (op. cit.) and Gallo (op. cit.) even found one full lethal limited to females. The main concern of the present work is with sex-limited genes in the Hymenoptera. This is not meant to imply, by any means, that sex-limited genes are the only source of the genetic variability of the Hymenoptera.

SEX-LIMITED VISIBLE AND STERILITY GENES

Three genes were found in a population of the social bee *Melipona marginata*, in 1945 (Kerr, 1951). One of these three genes was limited to the females (33%). A recent review of the genes for *Apis mellifera* (Rothenbuhler et al., 1968) showed that 5 (be, e, r, tr, u) out of 35 genes are totally or partially female limited (14.3%). The same is obviously true for many behavioral traits. This number of five does not include the sterility genes which have not been well studied in bees, and the genes for coloration of thorax and abdomen (Robert, 1951) which become limited to the females in *Apis mellifera adansonii* in the presence of gene *Ac* (Kerr, 1969); *Ac* is the only gene male-limited found so far in *Apis mellifera*. Genes which determine sterility in females but not in males are 35.9% of the total described genes (Saul et al., 1965) in *Mormoniella vitripennis*.

Martin (1947) cites 99 characters in *Bracon hebetor* that probably are controlled by 93 loci; with the intersex gene (*i*) (von

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Borstel and Smith, 1950) that makes 94. Of these, 4 (*pt*, *du*, *i*, *gy*) are totally or partially limited to the males (4.3%), and 20 (*gl*, *el*, *be*, *cr*, *pb*, *ho*, *m*, *sh*, *n*, *wa*, *sp*, *bw*, *gb*, *cw*, *ew*, *sl*, *ac*, *b*, *tw*, *cl*) are limited to the females (21.2%). Two of the male-limited genes are *i* and *gy*, which may be alleles of normal maleness genes (*i*⁺ and *gy*⁺). Martin (1947) cites the fact of nine intersexual females (all sisters), that tried to mate with normal females. This may indicate the expression of a femaleness allele.

Gonçalves (1970) studying 11 quantitative characters in *Apis mellifera* found that the genetic variances of five of them (45.7%) indicate that the genes responsible for their variability were predominantly female sex-limited.

SEX-LIMITED GENETIC LOAD

Chromosomal inversions cannot be applied in bees in order to detect the genetic load. However, using the method of Morton, Crow and Muller (1956), it was possible to estimate the lethal equivalents of four populations of *Apis mellifera*. The formulae proposed by those authors are also good for studying the genetic load using female Hymenoptera and are

$$S = e^{-\sum x - F \sum q s - (1-F) \sum q^2 s - 2(1-F) \sum q(1-q) s h}$$

making $A = \sum x + \sum q^2 s + 2 \sum q(1-q) s h$.

A (the intercept) biologically estimates the detrimental effects of the environment (M) and panmixia (P)

$B = \sum q s - \sum q^2 s - 2 \sum q(1-q) s h$, that is, B is equal to the genetic load (G) minus the effect of panmixia (P). Therefore, $S = e^{-(A + BF)}$ and the values of A and B may be obtained by a regression on: $-\log_e S = A + BF$. Since *Apis mellifera* females are heterozygous for a series of eleven sex alleles ($x_1 x_2$, $x_4 x_{11}$, etc.), Morton (1974) devised a modified formula which coped with this series, which is:

$$S = \left[\frac{1}{N} + \left(1 - \frac{2}{N} \right) (1 - F) \right] e^{-(A + BF)}$$

where N in the number of x alleles.

TABLE 1. Survival, from eggs to 15 days old pupae, of males of *Apis mellifera* ligustica, from a population kept for three years in Parada Neves, State of Espirito Santo, Brazil.

Number of the queens	F of the queen	S of the males
1 - A	0	0.689
6-2-61	0	0.887
7 - A	0	0.836
6-2-61	0	0.793
11-2-61	0	0.609
15-A	0.25	0.659
16-B	0	0.681
16-3-61	0	0.732
17-A	0	0.673
19-A	0	0.912
24-2-61	0	0.664
29-2-61	0	0.845
34-2-61	0	0.702
37-A	0	0.876
20-A	0	0.852
40-A	0	0.736
42-A	0	0.754
43-A	0	0.862
44-A	0	0.852
45-A	0	0.920
47-A	0	0.769
49-A	0	0.799
77-A	0	0.886
Total		17.988

$$\bar{S} = 0.782; -\log_e \bar{S} = 0.2459.$$

In the case of males, since they are haploid, and represent the development of a female gamete, these same values of A and B become: $A = \sum x$, that is, all deaths caused by the environment (M); $B = \sum q s$, that is, the direct value of the genetic load (G). No regression calculation can be applied since $-\log_e S = A + B$, which is an undefined equation. If an extrapolation for $F = 1$ is made for females, $-\log_e S = A + B = (M + P) + (G - P) = M + G$, and for males it is directly: $-\log_e S = A + B = M + G$.

Therefore, a coefficient of sex limitation L_s , can be obtained:

$$L_s = \frac{(A + B) \text{ of females}}{(A + B) \text{ of males}}$$

where, theoretically, if the genetic load is made up of genes which are not limited to

TABLE 2. *Survival from eggs to 15 days old male pupae, in a population of Apis mellifera mellifera, of Rio Claro, Brazil.*

Number	Number of eggs	Number of pupae	Survival
8-A	209	175	0.837
63-A	709	576	0.812
26-A	268	214	0.798
52-A	548	407	0.743
37-A	1106	444	0.457
19-A	120	101	0.842
21-Ju	330	301	0.912
44-A	526	454	0.863
10-A	678	655	0.966
62-A	560	506	0.921
Total			8.151

$$\bar{S} = 0.8151; -\log_e \bar{S} = 0.2046.$$

any sex, a value around 1.0 should be obtained. Where the genes which compose the load are limited to males, L_s should be smaller than one, and if they are limited to females, it should be greater than one.

The data (A and B) for females which are going to be used here are those of Kerr (1974b; Tables 1, 2, 3 and 4). From that paper the following figures were extracted:

a) *Apis mellifera ligustica*, population from Guarapari, E. S. Brazil, data in Table 1 of Kerr (1974b): $A = 0.169$, $B = 1.290$ with the x-alleles; or $A = 0.074$, $B = 0.262$ not counting the x-alleles.

b) *Apis mellifera mellifera*, population from Piracicaba, S.P., and Rio Claro, S.P., Brazil, data condensed in Table 2 of Kerr (1974b): $A = 0.182$, $B = 1.358$, counting the x-alleles; $A = 0.087$, $B = 0.311$ not counting the x-alleles.

c) *Apis mellifera adansonii* with some mixture with *A. m. ligustica* and their hybrids, population from Ribeirão Preto, S.P. Brazil, data in Table 3 of Kerr (1974b): $A = 0.115$, $B = 1.320$ counting the 11 x-alleles, and $A = 0.035$, $B = 0.192$, not counting these 11 x-alleles.

d) *Apis mellifera ligustica*, population from Chiba, Japan data taken from six well detailed graphs published by Hachinohe and Jimbu (1958); estimation of A and B in Kerr (1974b, Table 4): $A = 0.066$;

TABLE 3. *Survival from eggs to 15 days old pupae of males of a population of Ribeirão Preto, Brazil, which had a majority of African bees but also some Italian and hybrid bees.*

	Number of the queens	Survival of males (S)
1	24-1-67	0.9111
2	18-1-66	0.7661
3	50-1-66	0.9410
4	67-1-66	0.7936
5	19-1-66	0.9718
6	5-1-67	0.9020
7	R7-1-66	0.9412
8	180-1-67	0.7586
9	63-2-66	0.7791
10	R3-1-66	0.9247
11	R1-1-66	0.9234
12	R13-1-66	0.7448
13	135-1-66	0.7874
14	171-1-66	0.9677
15	84-1-66	0.4666
16	23-1-67	0.8790
17	R2-1-66	0.8745
18	83-5-68	0.9184
19	54-7-68	0.9652
20	R10-1-66	0.9420
21	7-1-66	0.9310
22	6-1-66	0.9412
23	148-1-67	0.9692
24	47-1-67	0.9783
25	137-1-67	0.9735
26	162-2-67	0.7830
27	43-4-68	0.8042
Total		23.5386

$$\bar{S} = 0.8718; -\log_e \bar{S} = 0.13697.$$

$B = 1.565$, counting the x-alleles; $A = 0.029$, $B = 0.545$ not counting the x-alleles. As an average value for N (number of x-alleles) $N = 11$ was taken. (see Kerr, 1967b).

The data for males are in Tables 1, 2, 3 and 4 of this work, which refer, respectively, to the same populations mentioned above, of the subspecies *ligustica*, *mellifera*, *adansonii*, and *ligustica* (Japan).

The multiple-allelic sex gene (eleven to twelve x-alleles) acts in the population of *Apis mellifera* as a true balanced sex-limited lethal, since all $x_1, x_2, x_3, \dots, x_{12}$ are viable haploid males, the homozygous diploids $x_1x_1, x_2x_2, \dots, x_{12}x_{12}$ are diploid males which, if reared with the techniques

TABLE 4. *Survival of males, from eggs to imago, from eight colonies of Apis mellifera ligustica, daughters of imported queens, established in Chiba, Japan. Data from Hachinohe and Jimbu (1958).*

	s
1	0.930
2	0.901
3	0.899
4	0.872
5	0.950
6	0.950
7	0.880
8	0.872
	7.254

$$\bar{S} = 0.9067; -\log_e \bar{S} = 0.09761.$$

developed by Woyke (1969) are viable but practically sterile; but if left in the hive, they are eaten by workers before the third day of larval life (Woyke, 1963). Therefore, due to these facts, each gamete has a gene that under the method of Morton et al. is detected as a full lethal. It contributes to B with $1 - 1/N$ and to A with $1/N$. In this way, two coefficients of sex limitation L_s may be estimated for each population; one with the eleven x-alleles in and one with the x-alleles out.

With all this in mind, the following coefficients of limitation to the sex can be obtained for *Apis mellifera* using the values of B and A obtained by Kerr (1974b):

a) Population of Guarapari. With the effect of the x-locus: $B = 1.290$; $A = 0.169$.

$$L_s^1 = \frac{A + B(\varphi)}{-\log_e S \delta} = \frac{1.459}{0.246} = 5.935.$$

Without the effect of the x-locus ($B = 0.262$; $A = 0.074$; $-\log_e S \text{ male} = 0.246$): $L_s^2 = 1.367$.

Therefore, 1 gene, 100% limited to the female sex, as any x-allele is, if taken out decreased the L_s coefficient of 4.568. If no sex-limited genes existed, L_s would be 1.0. Since the neutral point is 1, the fraction 0.367, out of 1.367, is sex-limited;

therefore we can say that 4.568 corresponds to 100% sex-limitation and therefore 0.367 will correspond to 8.04% of sex-limitation in the load of $B = 0.262$.

b) The population of Piracicaba and Rio Claro ($B = 1.358$; $A = 0.182$; $-\log_e S \text{ male} = 0.205$) produced a $L_s^1 = 7.527$ counting the x-alleles, and without the effect of the x-alleles ($B = 0.311$; $A = 0.087$), $L_s^2 = 1.94$, what means that 5.586 indicates 100% sex-limitation, and 0.942 would indicate 16.856% of sex-limitation in the $B = 0.311$ load.

c) The population of Ribeirão Preto ($B = 1.320$; $A = 0.115$; $-\log_e S \text{ male} = 0.137$) produced a $L_s^1 = 10.479$ and with no x-allele effect, L_s^2 of 1.657 ($B = 0.192$; $A = 0.035$) indicating that 7.5% of the non sex allele load ($B = 0.192$) is sex-limited.

d) The Japanese population studied by Hachinohe and Jimbu (1958) ($B = 1.565$; $A = 0.066$; $-\log_e S \text{ male} = 0.0976$), produced a $L_s^1 = 16.7065$ and, with no x-alleles effect ($B = 0.545$; $A = 0.029$) a $L_s^2 = 5.882$, and a female sex-limitation of 45.12%.

For estimating an average value of female limitation, we decided in favor of a balanced average, where the weight was the number of non-related queens used to estimate the value of B , added to the number of hives used to estimate the survival of males (Table 5). This balanced average (2501.4/185) of the four percentages of sex-limitation is 13.5% which grossly indicates that, in bees, about one-seventh of the genes which contributes to the genetic load are, to some extent, limited in their deleterious effects to the females. This per cent of sex-limited alleles is similar to those estimated for visible alleles in *Apis mellifera* (14.3%), sterility genes in *Mormoniella vitripennis* (35.9%) and *Bracon hebetor* (21.2%), to quantitative effects in *Apis mellifera* (45.7%).

SUMMARY

Evidence is presented which indicates that sex-limited alleles are of importance

TABLE 5. Estimation of the balanced value of the percentage of sex-limitation.

Place of the population studied	Number of hives in which male countings were made	Number of hives in which female countings were made	Total number of hives (T)	Per cent of non sex allele load that is sex limited	TX P
Guarapari	23	30	53	8.041	426.19
Piracicaba	10	46	56	16.856	943.94
Ribeirão Preto	27	34	61	7.453	454.63
Japan	8	7	15	45.107	676.61
			185		2501.37

$$\text{Balanced \% of sex-limitation} = \frac{2501.37}{185} = 13.52\%.$$

for the genetic variability of the Hymenoptera. A coefficient for sex limitation is formulated; such a coefficient indicates that about 14% of the detrimental alleles in *Apis mellifera* are limited to females.

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