

GENETIC ASPECTS OF ARTIFICIAL INSEMINATION

J. WOYKE

Thanks to artificial insemination, tremendous progress in bee genetics and bee breeding has been made. Many mutants have been discovered and are maintained. Knowledge of their inheritance helps to solve many genetical and physiological problems.

The origin of unusual bees and sex determination have been clarified. Progress has been made in breeding bees that are resistant to diseases, better pollinators, and better honey producers. These works can serve as guides in other fields of bee breeding.

Mutants

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More than 15 eye-colour mutants are known: *Mikhailoff* (1931) described a white eye mutation and studied its inheritance; *Rothembuhler*, *Gowen* and *Park* (1952) described ivory (*i*), cream (*cr*), snow (*s*), and chartreuse (*ch*) eyes (See table 5, p. 102). *Laidlaw*, *Green* and *Kerr* (1953) distinguished two chartreuse genes, ch^1 and ch^2 , and found red (ch^r), which is allelic to *ch*, and a non allelic gene, brick (*bk*). Interaction of *bk* and *ch* results in a buff phenotype. *Cale*, *Gowen* and *Carlile* (1963) found pink eye colour (*p*) which is also a viability gene. *Laidlaw*, *el Banby* and *Tucker* (1964) described five new eye-colour mutants: Benson green (ch^B), cherry (ch^c), garnet (*g*), pearl (*pe*) and tan (*s'*). Benson green and cherry are assignable to the chartreuse locus. Tan is allelic with *s* (snow); the *s/s'* genotype results in a peculiar phenotype which is red. Tan is epistatic to chartreuse 2, and to brick, but hypostatic to ivory and cream. Lately *Laidlaw* and *Tucker* (1965) described umber (i^u) which is an eye colour allele of ivory and partially dominant to it.



Fig. 56 — Albino drones (a) with unpigmented and non-sclerotized integument, but with normal eye pigment. This characteristic feature is recessive, and no mature spermatozoa have been obtained (Hutterer, unpublished)

The eye colour mutants are slightly different in haploid drones and diploid females. Woyke (1973) provided experimental evidence that this effect is not caused by gene differences as it had been assessed, because both haploid and diploid drones have mutant eyes of the same colour.

All these genes are recessive to wild type. Linkage was found between pearl and cream with a 0.33% crossing over value (Laidlaw, et al Banby and Tucker 1965). Linkage of eye-colour mutants and some other mutants were also found. Mackensen (1958) described the linkage between chartreuse and hairless; Laidlaw, et al Banby and Tucker (1965) mention the possibility of linkage between brick and reduced facet number. Woyke (1971) described laranja (*la*) mutant, which is epistatic to brick and combines with chartreuse resulting in a buff colour.

Three eye shape mutants were also described: Cyclops (Lotmar 1936), reduced facet number (*rf*; Kerr and Laidlaw, 1956) and eyeless (*e*; Laidlaw and Tucker 1965). The eyeless drones are sterile and no testes were found.

Very useful as marker is the body colour mutation cordovan (*c*), described by Mackensen (1951). The black gene (*bl*) described by Laidlaw and et al Banby (1962) suppresses the yellow colour of the Italian bees in homozygotes and hemizygotes.

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Fig. 57 — Bee with cyclop eye. The two compound eyes have merged into one only — in the middle of the head. Simple eyes do not exist. Queens with such eyes have also been found. This feature was transmitted infrequently for 5 successive generations (0.1—2% in workers; *Ruttner*, unpublished)



Fig. 58 — Dominantly inherited hairlessness (H). The long hairs are missing over the entire body but the short hair bands on the abdomen are present. The bee to the right of center in the illustration has normal hair. The character is inherited as a monofactorial dominant. The mutant drones are not viable. (*Ruttner* unpublished)

The body hair mutations, erbliche Schwarzsucht (*S*; Dreher 1940) and hairless (*h*; Mäckensen 1957) are described. Hairless is linked with *ch* (4.1% crossing over; Mackensen 1958).

Five wing mutations were found: Rudimental wing (*Rw*; Hachinohe and Onishi, 1953), Droopy (*D*; Rothenbühler, Gowen and Park, 1952), short wing (*sh*; Kerr and Laidlaw, 1956), and truncate (*tr*) and wrinkled wing (*wr*; Laidlaw, el Banby and Tucker, 1965). These mutations are mostly linked with lethals, or are semilethal. *Rw* and *i* are linked with 31% crossing over (Hachinohe and Onishi 1963). Several other mutants have been recorded but not yet published (See Table 5).

Unusual Bees (Mosaic and Gynandromorph Individuals)

Artificial insemination together with the mutant genes used as markers, has made it possible to determine the origin of unusual bees (Tab. 4).

Different unusual bees originating from *unfertilized* eggs have been described. Unfertilized binucleate eggs result in mosaic males (Tab. 4, 1 a) (Tucker 1958; Woyke 1962). Uniting of two pronuclei in unfertilized eggs produces parthenogenetic females (Tab. 4 1 b). Two haploid egg pronuclei can divide at least once before union. Next two haploid nuclei unite to form a diploid cleavage nucleus which develops into female tissues; the other haploid nuclei develop into mosaic male tissues (Tab. 4, 1 c). Thus a gynandromorph is formed (Fig. 59).

Different individuals originating from inseminated eggs have been described :

From *fertilized* eggs, not only females can develop, as it generally happens, but, when the sex alleles are in common, diploid males develop as well (Woyke 1963). More details about these will be given in the next section.

If only one pronucleus of the two are fertilized in a binucleate egg, the developing gynandromorph possesses male tissue of matroclinous origin and the female tissues of biparental origin (Tab. 4, 2.1b). Polyspermy may be the cause of several types of unusual bees : Two sperms in an egg can unite, leaving the egg pronucleus unfertilized (Tab. 4, 2.2a). This results in a gynandromorph in which the diploid female tissues originate from two different fathers, without the participation of the mother. Polyspermy of an egg with one nucleus results in a fertilization of the nucleus but, sometimes, one or more accessory sperms do not degenerate and develop into male tissues. Thus a gynandromorph develops with the

ORIGIN OF UNUSUAL HONEY BEES HITHERTO DESCRIBED

1. From uninseminated egg



Mosaic male

Tucker 1958, Woyke 1962



Parthenogenetic female

Mackensen 1953, Tucker 1958,
Woyke 1962, Tryasko 1965



Gynandromorph with mosaic
male tissues

Tucker 1958

2. From inseminated egg

2.1. One sperm takes part in the origin of a bee.



Diploid male

Woyke 1963, 1965



Gynandromorph with matrocli-
nous male tissues

Mackensen 1951, Woyke 1962,
Drescher, Rothenbuhler 1963

2.2. More sperms take part in the origin of a bee.



Gynandromorph with di-
ploid female patroclinous
tissues

Laidlaw, Tucker 1964



Gynandromorph with patro-
clinous male tissues.
Mosaic male with some di-
ploid tissues

Rothenbuhler, Gowen, Park
1952, Drescher, Rothen-
buhler 1963



Mosaic female

Taber 1955, Woyke 1962



Mosaic female with diploid
parthenogenetic tissues

Woyke, 1962

sperm ● egg pronucleus  or  zygote



Fig. 19 — Head of gynandromorph bee. The right part of the body is male, the left — worker (Photo by Rothenbuhler)

origin of tissues different from those mentioned above. Here the female diploid parts are originating from both parents but the haploid male parts develop from sperm only (Tab. 4, 2.2 b).

A mosaic female can result from polyspermy in a binucleate egg if sperms of different fathers unite with the two pronuclei (Tab. 4, 2.2c). The two haploid egg pronuclei can first divide and then two of them can be fertilized by different sperms and the other two can unite resulting in a female that is partly parthenogenetic and partly has two fathers (Tab. 4, 2.2 d).

Sex Determination

The problem of sex determination could be solved only thanks to artificial insemination. *Mackensen* (1951) found out that, after individual mother daughter matings (p. 110) one half of the queens produced brood in which only 50% of the individuals survived. This was confirmed by *Hachinohe* and *Jimbu* (1958). Later *Mackensen* (1955) found at least 11 different alleles responsible for this effect. *Laidlaw*, *Gomes* and *Kerr* (1956) found 12.4 ± 3.56 such alleles in a panmictic population in Brazil. *Rothenbuhler* (1957) reported patches of diploid male tissue in eyes of mosaic drones from related parents of a gynandromorph line. This was confirmed later by *Drescher* and *Rothenbuhler* (1964). But a drone of completely zygotic origin was not found. It was believed that the homozygous eggs for an "x" locus, obtained by inbreeding did not hatch, and that small areas of diploid male tissue could survive only by virtue of their association with normally viable haploid male tissue.

But *Woyke* (1962) showed in the meantime that all the eggs from inbred queens hatch. He proved that some drone larvae are of completely zygotic origin (1963). The low survival rate is caused by the nurse bees eating newly hatched larvae (1963). The diploid drone larvae are viable and can be reared to imagines (*Woyke* 1963, 1965). Their origin from fertilized eggs was proved cytologically as well as genetically (*Woyke* 1965 ; *Woyke* and *Knytel* 1966).

It can be stated that a series of sex alleles exists which, in heterozygotes, results in females, and in hemizygotes and homozygotes, in males. The homozygotes are not inviable but are eaten by worker bees, and therefore were not observed.

Bee Diseases

Resistance of honey bees to disease had already been reported before artificial insemination was used in disease resistance studies (*Sturtevant* 1920 ; *Parke*, *Pellett* and *Paddock*, 1937). It was concluded that resistance to American foulbrood is heritable. Also, some information had been gained on the mechanism of resistance to European foulbrood (*Sturtevant* 1920) as well as American foulbrood (*Woodrow* 1941 ; *Woodrow* and *Holst* 1942 ; *Sturtevant* and *Revel* 1953).

Artificial insemination has made more detailed studies possible. *Rothenbuhler* and *Thompson* (1956) found highly significant differences between lines in survival of larvae treated with American foulbrood spores. This resistance is also heritable (*Lewis* and *Rothenbuhler* 1961). When the larvae of two lines had received spores at 21 hours of age,

differences were found both in time of germination and in the number of bacteria observed (*Bamrick* 1964). But also the adult bees of different genetic lines protect the larvae to different degrees (*Thompson and Rothenbuhler* 1957).

A strong difference in behaviour of four inbred lines toward brood dead of American foulbrood was demonstrated by *Rothenbuhler* (1964). Two inbred lines of bees showed a great difference in the time required to uncap and remove cyanide-killed brood (*Jones and Rothenbuhler* 1964). Colonies composed of young resistant bees will remove all larvae killed by foulbrood, whereas colonies made up of bees older than about 4 weeks remove the larvae only during a nectar flow. After genetic differences had been found, the heredity could be investigated. *Rothenbuhler* (1964) made the necessary crosses and found, in the backcrosses, four types of colonies: 1) Uncappers of cells and removers of dead brood contained therein, 2) uncappers only, 3) removers only after human uncapping, 4) neither uncappers nor removers. He developed a two locus hypothesis. It states that uncapping of a cell containing dead brood depends upon homozygosity for a single recessive gene (designated *u*) and removing depends upon homozygosity for a second single recessive gene (designated *r*).

Drescher (1964) found that the tendency to paralysis (*Schwarzsucht*) was irrespective to the mating drone. He suggested a matroclinal heredity.

Pollination

Differences in pollen collection of colonies have often been observed and studies have been made to determine if such differences are hereditary. *Nye and Mackensen* (1965) made studies to determine if the tendency to prefer alfalfa pollen is inherited. They showed that colonies headed by sister queens were more similar in the proportion of alfalfa pollen collected than those headed by unrelated queens. This suggested heritability. Later (*Mackensen and Nye* 1966, 1969; *Nye and Mackensen* 1968) they were able to select low and high preference lines, the selection being based on the percentage of pollen collectors that were collecting alfalfa

pollen. In the high preference line, this percentage increased from 39.8 in the second generation to 86 in the sixth. In the line showing low preference the corresponding percentages were 26.2 and 8. From crosses and backcrosses they concluded that this characteristic is probably controlled by several genes that have a mainly additive effect.

These results prove beyond doubt that the tendency to collect alfalfa pollen in preference to other pollens is heritable. They open up the possibility of developing special high quality strains or hybrids of honey bees for commercial use in pollinating alfalfa. Probably it is also possible to breed strains of bees better capable of pollinating red clover and other crops.

LIST OF MUTATIONS IN THE HONEYBEE

Sym- bol	Name of mutation	When named and by whom	Aspect	Characteristics
	Eye colour			
—	white	1931 <i>Mikhatoff</i>	white	
<i>bk</i>	brick 1	1953 <i>Laidlaw, Green, Kerr</i>	newly emerged bee (ne.) brick-red; later on (lo.) red brown	<i>bk</i> , <i>ch</i> ² interact buff, <i>bk</i> , <i>chr</i> and <i>bk</i> , <i>chc</i> interact pink (<i>Laidlaw</i> et al. 1953, 1964), exist semilethal, hypostatic to <i>t</i> , to <i>cr</i> , and to <i>s</i> (<i>Mackensen</i> 1958), hypostatic to <i>s</i> ^t (<i>Laidlaw</i> et al. 1964)
<i>by</i>	bayer	<i>Laidlaw</i> (unpublished work)	ne. white, lo. reddish-orange	No allele to <i>bk</i> , <i>ch</i> , <i>cr</i> , <i>g</i> , <i>i</i> , <i>pe</i> , <i>sp</i>
<i>ch</i>	chartreuse	1952 <i>Rothembuhler, Gowen, Park</i>	ne. yellow green, lo. olive green — reddish to reddish brown	⁽¹⁾ hypostatic to <i>i</i> , to <i>cr</i> and to <i>s</i> (<i>Rothembuhler</i> et al. 1952), linked to <i>h</i> , crossover 4.1 (<i>Mackensen</i> 1958)
<i>ch</i> ¹	chartreuse-1	1953 <i>Laidlaw, Green, Kerr</i>	like <i>ch</i> , — slightly darker brown (variable)	allele to <i>ch</i> , affected by <i>m</i> and interact brown, <i>ch</i> ¹ / <i>chr</i> intermediate (<i>Laidlaw</i> et al. 1953), recessive to <i>chc</i> , dominant to <i>ch</i> ^B (<i>Laidlaw</i> et al. 1964)
<i>ch</i> ²	chartreuse-2	1953 <i>Laidlaw, Green, Kerr</i>	like <i>ch</i> , greener, lo. reddish to reddish brown	allele to <i>ch</i> , <i>ch</i> ² / <i>chr</i> intermediate, <i>ch</i> ² , <i>bk</i> interact buff, hypostatic to <i>t</i> and to <i>s</i> ^t (<i>Laidlaw</i> et al. 1953, 1964)

Sym- bol	Name of mutation	When named and by whom	Aspect	Characteristics
<i>chB</i>	Benson green	1964 Laidlaw, el Banby Tucker	like <i>ch</i> ² , but ne. ♂♂ greener lo. olive green to reddish	allele to <i>ch</i> , recessive to <i>ch</i> ¹
<i>chc</i>	cherry	1964 Laidlaw, el Banby Tucker	worker bees dark red, ♂♂ yellow to red brown (very variable)	allele to <i>ch</i> , dominant to <i>ch</i> ¹ , <i>bk</i> ; <i>chc</i> interact pink
<i>chr</i>	red	1953 Laidlaw, Green, Kerr	ne. purple red, lo. red brown	allele to <i>ch</i> ¹ / <i>chr</i> and <i>ch</i> ² intermediale; <i>chr</i> ; <i>bk</i> interact pink, hypostatic to <i>i</i> (Laidlaw et al. 1953)
<i>cr</i>	cream	1952 Rothenbuhler, Gouwen, Park	white	epistatic to <i>ch</i> (Rothenbuhler et al. 1952), to <i>bk</i> (Mackensen 1958) and to <i>s</i> (Laid- law et al. 1964), linked to <i>pe</i> , crossover 0.33 (Laidlaw et al. 1965).
<i>g</i>	garnet	1964 Laidlaw, el Banby Tucker	ne. garnet red, lo. dark even in wild type	
<i>i</i>	ivory	1952 Rothenbuhler, Gouwen, Park	white	epistatic to <i>ch</i> (Rothenbuhler et al. 1952), to <i>ch</i> ² and <i>chr</i> (Laidlaw et al. 1953), to <i>bk</i> (Mackensen 1958), and to <i>st</i> (Laidlaw et al. 1964), partly recessive to <i>iw</i> (Laid- law et al. 1965).
<i>iro</i>	rose	Laidlaw (unpublished work)	ne, clear rose pink, lo. pink	homozygous ♀♀ do not fly to mate
<i>iu</i>	umber	1965 Laidlaw, Tucker	ne. peach pink; lo. yellow- reddish-brown	allele to <i>i</i> , partly dominant to <i>i</i>
<i>la</i>	laranja	1973 Woyke	clear orange, lo. reddish brown	epistatic to brick, <i>la</i> / <i>ch</i> interact buff (Woyke 1973)

Sym- bol	Name of mutation	When named and by whom	Aspect	Characteristics
<i>m</i>	modifier	1953 Laidlaw, Green, Kerr	brownish at <i>ch</i> ¹ -animals	affect <i>ch</i> ¹ , <i>ch</i> ¹ / <i>m</i> interact brown
<i>p</i>	pink	1963 Cale, Gowen, Carlike	rose pink	partly semilethal
<i>pe</i>	pearl	1964 Laidlaw, el Banby, Tucker	white	linked, to <i>cr</i> , crossover 0.33 (Laidlaw et al. 1965)
<i>s</i>	snow	1952 Rothenbuhler, Gowen, Park	white (cannot be distinguished from ivory, cream and pearl)	epistatic to <i>ch</i> (Rothenbuhler et al. 1952), and to <i>bk</i> , exist semilethal (Mackensen 1958)
<i>sp</i>	spade	Laidlaw (unpublished work)	ne. rose pink. lo. red (similar to <i>bk</i>)	no allele to <i>bk</i> , <i>ch</i> , <i>cr</i> , <i>g</i> , <i>i</i> , <i>pe</i> , <i>by</i>
<i>st</i>	tan	1964 Laidlaw, el Banby Tucker	ne. white; lo. bright yellow-brownish	allele to <i>s</i> , <i>s</i> / <i>st</i> is red, epistatic to <i>ch</i> ² and <i>bk</i> , hypostatic to <i>t</i> and <i>cr</i>
—	Eye shape	1936 Lotmar	Cyclops eyes	dominant, transmitted infrequently via eggs (Lotmar 1936, Kerr and Laidlaw 1956, Laidlaw et al. 1965).
<i>rf</i>	reduced facet number	1956 Kerr, Laidlaw	atrophied eyes owing to small number of facets	inherited complex in low frequency with <i>bk</i> or <i>g</i> (Laidlaw et al. 1965)
<i>e</i>	eyeless	1965 Laidlaw, Tucker	no facets	Male sterile, semilethal in hemizygote

Sym- bol	Name of mutation	When named and by whom	Aspect	Characteristics
	Body pigment			
a	albino	Ruttner (unpublished work)	non-pigmented integument, non-sclerified, normal eye pigment (Fig. 56)	incomplete spermatogenesis, semilethal
c	cordovan	1951 Mackensen, Nolan	colour similar to that of leather	
bl	black	1962 Laidlaw, et Banby	dark colour — Italian bees	
	Body hairs			
S	schwarzstüchtig	1940 Dreher	no down	dominant to wild type
h	hairless	1958 Mackensen	no down	recessive to wild type, semilethal to dro- nes, linked to <i>ch</i> , crossover 4.1
H	Haarlos	Ruttner (unpublished work)	No hairs, hair on tomentum exists (Fig. 58)	Bristles (pollen rake) are brittle, the viable heterozygous workers produce small pellets. Hemizygotes (♂♂) are lethal
	Wings			
d†	diminutive	1967 Laidlaw	Small wing, normal venation	When flying workers and ♂♂ produce a high tinkling sound. Homozygous ♀♀ do not fly (Witherell, 1973)
D	Droopy	1952 Rothenbuhler, Gowen, Park	flat, separated wings, cannot fly	dominant to wild type, lethal in hemi- zygote and homozygote.

Sym- bol	Name of mutation	When named and by whom	Aspect	Characteristics
<i>Rw</i>	Rudimental wing	1953 Hachinohe, Onishi	atrophied wings	dominant to wild type, linked tot <i>l</i> , crossover 31
<i>sh</i>	short	1956 Kerr, Laidlaw	small wing, bees cannot fly, abnormal venation	semilethal (Laidlaw et al. 1965)
<i>tr</i>	truncate	1965 Laidlaw, el Banby Tucker	the wing looks as if cut in the middle, bees cannot fly, abnormal venation	semilethal
<i>wr</i>	wrinkled	1965 Laidlaw, el Banby	"rumped" wing	incomplete penetrance; increase by com- bination with <i>bk</i>
<i>l</i>	lethal	1958, Hachinohe, Onishi	dead in early stage	linked with <i>Rw</i> , crossover 31

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