AUTOMICTIC PARTHENOGENESIS IN THE HONEY BEE[1]

KENNETH W. TUCKER[2]

Department of Entomology and Parasitology, University of California, Davis
Received May 15, 1957

IN the honey bee (*Apis mellifera* L.), fertilized eggs (diploid zygotes) regularly develop into females, and unfertilized eggs (haploid gametes) develop into males by types of reproduction known as zygogenesis and generative or haploid parthenogenesis, respectively. The occasional appearance of females among the male progeny of unmated queens and laying workers is therefore of considerable interest. This phenomenon has been known for many years (HEWITT 1892; ONIONS 1912, 1914; JACK 1917; MACKENSEN 1943; FYG 1949; BUTLER 1954); however it is usually undetected and has not been satisfactorily explained.

That impaternate queens are diploid is indicated by the fact that they reproduce normally (MACKENSEN 1943; BUTLER 1954). SUOMALAINEN (1950) designates diploid and polyploid parthenogenesis as somatic parthenogenesis.

One known case of somatic parthenogenesis in the honey bee (KERR unpublished) suggests automictic parthenogenesis. This type of somatic parthenogenesis requires meiotic chromosome reduction, as opposed to apomictic parthenogenesis in which meiosis is completely absent (SUOMALAINEN 1950). KERR obtained results denoting meiotic chromosome reduction; these were the appearance of two homozygous recessives among eight impaternate worker bees from heterozygous unmated queens.

Impaternate females have been found in the following races of the honey bee: Punic and Syrian (HEWITT 1892), South African Cape (ONIONS 1912, 1914; JACK 1917; see also critiques by WARMELO 1912; DADANT 1919; ANDERSON 1918; and BALDENSPERGER 1918), Italian (MACKENSEN 1943; BUTLER 1954), Caucasian (MACKENSEN 1943) and northern Russian (GUBIN and KHALIFMAN 1951). MACKENSEN (1943) estimated that impaternate females did not exceed one percent of the progeny of any one unmated queen in the three strains he investigated.

The primary aim of the present study was to determine the cytological basis for somatic parthenogenesis in the honey bee. Cytological mechanisms were inferred from the segregation of mutant genes, since impaternate females were too scarce to insure adequate material for a microscopic examination. The secondary purpose of this work was to determine the comparative frequency of impaternate females among different lines.

Selection and use of mutants

The segregation of mutant genes and their distribution among the worker progeny of unmated queens heterozygous for these genes should provide clues to the mechanism responsible for somatic parthenogenesis. Red (ch^r), chartreuse (ch^2), and ivory (i) eye color and cordovan (cd) body color mutants were chosen for this purpose because they are easily classifiable and are not affected by known modifiers. Red was used with its allele chartreuse in the hope that ch^2/ch^r workers could be distinguished from ch^r/ch^r workers. The recessive genes ch^2, i, and cd were used with their dominant wild type alleles. These genes segregate independently (LAIDLAW et al. 1953).

The only difficulty in classifying newly emerged impaternate workers was that ch^2/ch^r workers were often indistinguishable from ch^r/ch^r workers. Initially these phenotypes were believed to be distinct, pink for ch^2/ch^r and red for ch^r/ch^r; but a relative excess of workers classed "red" and a gradation of eye color from pink to red in those classed "pink" led to the suspicion that the genotypes were often confused with one another. This suspicion was corroborated by observations on eye colors of zygogenetic workers of known genotypes.

Production of impaternate workers

The most expedient methods of impaternate worker production which evolved during the study are given here. Manipulation procedures were adapted from LAIDLAW and ECKERT (1950) and LAIDLAW (1954).

Virgin queen honey bees were reared by standard methods and permitted to emerge in individual cages within a nursery colony. On each of two successive days, usually the sixth and seventh after emergence, they were anaesthetized with carbon dioxide for ten minutes to hasten the initiation of oviposition (MACKENSEN 1947). Their wings on one side were clipped to prevent mating.

The queens were then introduced into nuclei and confined by entrance queen excluders until they started to lay. They were usually supported with phenotypically distinguishable worker bees to enable detection of laying worker progeny (see MACKENSEN 1943). However, laying workers did not occur where queenlessness and broodlessness were avoided and never presented a serious source of error.

The unmated queens produced brood in worker comb and in drone comb. Drone comb provided higher viability of male progeny than worker comb and was used almost exclusively until sufficient data were obtained on the occurrence of impaternate workers.

When a comb was fairly well filled with brood, the oldest of which was usually four or five days old, it was removed from the nucleus and the brood reared to the sealed stage in a strong, queenless feeder colony. When sealed and/or no more than 20 days old, the combs of brood were removed from the feeder colony, freed of *all* adult bees, and caged in frame cages. The caged combs of brood were

placed in a humidified incubator at 92° F and left there until the brood was counted on the fifteenth day after removal from the nucleus, by which time the youngest drone brood would have pupated.

Laying workers occurred in a few colonies being used in another study as well as in a few of the nuclei used in these experiments. Brood produced by these laying workers was handled in the same manner as was brood from unmated queens.

Interrupting oviposition

Actively laying unmated queens were caged to interrupt oviposition. Most of those caged in their own nuclei continued to lay and dropped their eggs in the bottoms of their cages. Those caged in a nursery colony stopped laying if the nursery colony was not fed lavishly. Queens caged in the laboratory with food and attendant worker bees also ceased laying. After these trials, caging in the nursery colony was used exclusively since it was effective and the most convenient.

Counting technique

Counts were made first of adult bees if any had emerged, then of any brood which had pupated on the bottom of the frame cage, and finally of pupae in capped cells which were exposed by uncapping each sealed cell with a dissecting needle. Even when only workers were counted, all capped cells in worker comb and all flat-capped cells in drone comb were uncapped. Combs containing worker pupae were returned to the incubator, where the workers were permitted to complete their development and emerge.

All brood in the incubator were inspected daily, both before and after counts were made. More frequent inspections were made when workers were newly emerged; these workers were removed and classified immediately.

GENETIC RESULTS AND ANALYSIS

One percent or less of the progeny from unfertilized eggs of unmated queens and laying workers were exceptional bees, of which 97 percent were workers, two percent gynandromorphs, and one percent mosaic drones. The genetic analysis below proposes that these unusual bees all originate by similar rather than by different means.

Impaternate workers

Impaternate workers were probably normal diploids, since they were indistinguishable from zygogenetic workers in external morphology and behavior.

With the sole exception of impaternate workers from queens heterozygous for the cordovan gene, homozygous recessive workers were produced by heterozygous unmated queens (Tables 1 and 2). The absence of cd/cd workers may have been due to the small number of workers (13) produced by cd/cd^+ queens inasmuch as only one i/i worker occurred in a larger sample from i/i^+ queens. The percentage of cd/cd workers could be less than that of ch^z/ch^z workers, since at least

TABLE 1

Segregation in impaternate workers produced by unmated queens heterozygous for the genes indicated

Numbers and genotypes of queens tested	Phenotypes of progeny*				
	Normal	Red and pink	Chartreuse	Ivory	Cordovan
28 ch^z/ch^r		106	15		
3 $ch^z/ch+$, $i/i+$, $cd/cd+$	1		0	0	0
5 $ch^z/ch+$, $i/i+$	35		9†	1	
6 $i/i+$, $cd/cd+$	10			0	0
1 $i/i+$	1			0	
1 $cd/cd+$	2				0

* In addition, seven workers were lost and three died before emergence with ch^z/ch^r, and one worker died before emergence with $ch^z/ch+$, $i/i+$.
† This includes one gynandromorph.

TABLE 2

Summary of homozygosity, and comparisons to hypothetical segregation for the gene pairs indicated. Data from Table 1

Genic combinations	Total number of workers	Percentage homozygous recessive	Chi-squares to hypothetical segregation ratios (1 d.f.)			
			1:1	3:1	5:1	7:1
ch^z/ch^r	121	12.4	71.48†	12.00†	2.46	0.03
$ch^z/ch+$	46	19.6	17.06†	0.29	0.20	1.66
$i/i+$	57	1.8	53.09†	17.01†	9.88†	6.79†
$cd/cd+$	13	0.0	13.08†	5.10*	3.55	2.89

† Significantly different from expected at the one percent level.
* Significantly different from expected at the five percent level.

one ch^z/ch^z worker occurred in each consecutive subsample with 13 workers taken from the 167 workers produced by $ch^z/ch+$ and ch^z/ch^r queens.

The low proportion of i/i workers is probably not a reflection of selective mortality of this genotype. Although no selective mortality has been reported in i/i workers of zygogenetic origin (ROTHENBUHLER *et al.* 1953, table III), it could be possible in the female progeny of close relatives or in impaternate females if the ivory locus were closely linked to the lethal alleles (see MACKENSEN 1951). However, the following test failed to indicate selective mortality: two $i/i+$ sister queens, each outcrossed to one ivory-eyed drone, produced 47.4 percent (n=568) and 48.2 percent (n=721) i/i workers; a third $i/i+$ sister queen, selfed with one of her ivory-eyed drones, produced 48.7 percent (n=242) i/i workers; none of these proportions differ significantly from that expected without selective mortality (50 percent i/i).

The difference in the proportions of homozygosity obtained with ivory and chartreuse could be caused by different degrees of linkage between these loci and their respective centromeres. The influence of such linkage on the segregation is

consistent with several of the explanations of parthenogenesis which will be considered below.

The fact that homozygous recessive impaternate females appeared as progeny of heterozygous unmated queens indicates that allele segregation and recombination, hence automictic parthenogenesis, occurred. Such segregation and recombination could possibly be brought about in these general ways (SUOMALAINEN 1950; SPEICHER and SPEICHER 1938; STALKER 1954): (1) meiosis in tetraploid tissue, and (2) meiosis in diploid tissue with the diploid number either retained by unusual meiosis or restored by unusual union of haploid nuclei.

Meiosis in tetraploid tissue: The parthenogenetic production of a diploid cleavage nucleus by normal meiosis in tetraploid tissue only partly meets the requirements of SUOMALAINEN's (1950) definition of automictic parthenogenesis in that the chromosome number is doubled before meiosis rather than restored during or following meiosis. However, it is certainly not apomictic parthenogenesis—no meiosis—the only alternative SUOMALAINEN (1950) mentioned. Because it results in allele segregation and recombination, it will be considered a type of automictic parthenogenesis in this analysis.

The presence of patches of tetraploid tissue in the ovaries of *Habrobracon juglandis* females in parthenogenetic lines is suggested by a study of meiosis and early cleavage in eggs from these wasps (SPEICHER and SPEICHER 1938). In tetraploid eggs, normal meiosis of the diploid number of bivalents (rather than N quadrivalents) produces an egg pronucleus and the normal number of polar bodies, each of which is diploid rather than haploid. Normal cleavage of the diploid egg pronucleus produces a female. The appearance of 2N bivalents, rather than N quadrivalents, in metaphase I suggests that there is no exchange between homologous bivalents (DARLINGTON 1937, pp. 119–129). With random prophase pairing, 16.7 percent homozygous recessives would be expected. However, most of the genetic results in Habrobracon (SPEICHER 1934) were closer to 25 percent homozygous recessives, as would be expected if prophase pairing occurred only between maternal and paternal chromosomes. With neither sort of prophase pairing can the centromere influence the segregation of genes which are closely linked to it because each diploid nucleus should receive a single chromatid from each of the two homologous bivalents. In the present data, the proportion of ch^2/ch^2 workers fits the expectancies, especially to random prophase pairing, but the frequency of i/i workers is too low (Table 2). Tetravalent segregation could account for the low frequency of i/i workers if the ivory locus is close to its centromere and prophase pairing occurs only between maternal and paternal chromosomes. Since nonrandom prophase pairing is improbable (DARLINGTON 1937), it is unlikely that normal meiosis of tetraploid ovarial tissue can account for automictic workers in the honey bee.

Meiosis in diploid tissue: Following normal meiosis in diploid tissues, a diploid cleavage nucleus could be formed automictically by the union of the first two haploid cleavage nuclei (as in *Solenobia triquetrella*). A heterozygous queen could produce only homozygotes (50 percent recessives) because the first two

cleavage nuclei would come from the same egg pronucleus. The results of the present study cannot be explained on this basis since homozygotes are far too infrequent (Table 2, column under 1:1). Moreover, cleavage nuclei formed by this means should be homozygous for the lethal alleles and produce inviable embryos (MACKENSEN 1951).

A diploid cleavage nucleus could also be automictically produced by the recovery of two of the four chromatids of each segregating bivalent. A diploid cleavage nucleus could result if two of the four haploid nuclei produced by normal meiosis unite. These four nuclei are arranged in a row perpendicular to the surface of the egg so that two adjacent nuclei unite to form the diploid nucleus. Two kinds of union are possible: either terminal union, the union of the nucleus farthest from the egg's surface with its neighbor, or central union, the union of the two central nuclei in the row of four nuclei. For each segregating bivalent, terminal union recovers two chromatids from the same secondary oocyte, while central union recovers one chromatid from each of the two secondary oocytes. The same ends could be attained by abnormal meiosis. These types are the genetic equivalents of the terminal union of two haploid nuclei where the diploid cleavage nucleus is a secondary oocyte, or the genetic equivalents of central union of haploid nuclei where the diploid cleavage nucleus receives chromatids equally from each of the two secondary oocytes.

The genetic results expected with terminal union and with central union contrast sharply with extremes of equational separation of alleles in meiosis I. (Figure 1).

By superimposing the observed segregation of mutant genes (Table 2) on the expectancies shown in Figure 1, it seems probable that the ivory locus is closely
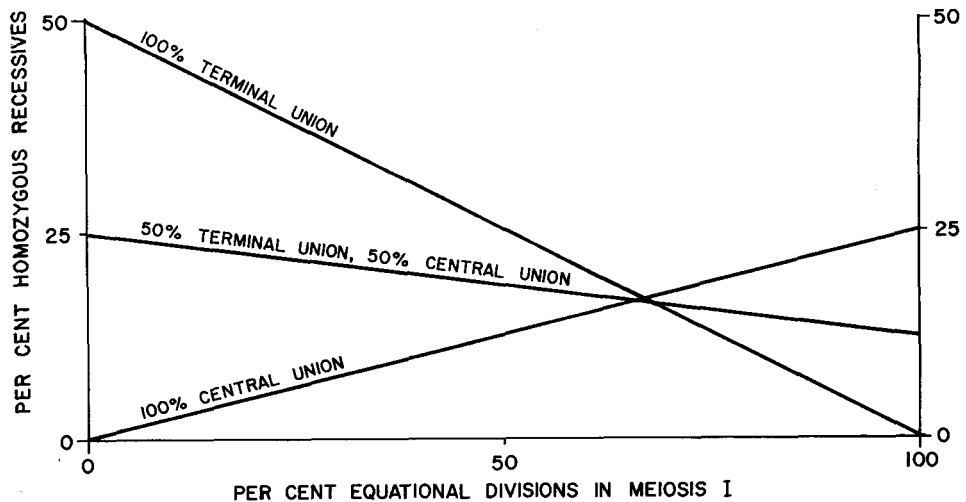

FIGURE 1.—Percentage of homozygous recessives expected in diploid progeny of heterozygous mothers with different schemes of union of haploid nuclei accompanied with various amounts of equational separation of alleles in meiosis I.

linked to its centromere and that the chartreuse locus is less closely linked to its centromere. Assuming that the centromere of a bivalent consistently divides either reductionally only and with 100 percent central union, or equationally only and with 100 percent terminal union, the proportion of i/i workers conforms to the segregation expected of a gene which is closely linked to its centromere. The proportion of ch^2/ch^2 workers conforms to neither extreme of the percentage of equational divisions in meiosis I, so the chartreuse locus must be less closely linked to its centromere.

Because exclusively equational division of the centromere in meiosis I seems unlikely, it is improbable that the observed segregation can be explained by a terminal union mechanism, such as occurs in *Neuroterus baccarum*, *Nemeritus canescens*, *Solenobia lichenella* and *Diprion polytomum*.

Assuming reductional division of the centromere in meiosis I, as most authors do (e.g., CATCHESIDE 1951; DARLINGTON 1937; MATHER 1935), the observed segregation favors a high proportion of central unions. The low proportion of i/i workers is decisive since a closely linked gene would be expected to yield a low proportion of homozygotes with 100 percent central union.

In one central union type, *Apterona helix*, the first division is normal, but the two second division metaphase plates unite. The division of the compound plate forms two diploid nuclei, both of which function as cleavage nuclei. A heterozygous queen could produce only heterozygotes and diploid mosaics. However, the existence of homozygotes and the absence of diploid mosaics in the present study cannot be explained by this mechanism.

Central union and terminal union, in about equal proportions, were indicated for parthenogenetic diploids in *Drosophila parthenogenetica* (STALKER 1954). However, the genetic expectancies of this combination (Figure 1) cannot be reconciled to the segregation of the ivory gene (Table 2).

In the honey bee the diploid polar union nucleus, which ordinarily degenerates, is formed by central union of the central half of the first polar body and the second polar body (PETRUNKEWITSCH 1901; NACHTSHEIM 1913). But if the polar union nucleus develops into female tissue, the egg pronucleus might be expected to develop into male tissue, and only gynandromorphs would be formed. However, the rarity of gynandromorphs suggests that either the development from the egg pronucleus is somehow suppressed or that the genetic equivalent of central union is accomplished in some other way.

Another way to achieve the genetic equivalent of central union is the spindle misorientation scheme (Figure 2). Ordinarily the first meiotic spindle in a newly-laid, honey bee egg lies in a sagittal plane parallel to the surface of the egg. The spindle soon rotates through a 90° angle so that by the end of the first division it projects into the egg perpendicular to the egg's surface (PETRUNKEWITSCH 1901; NACHTSHEIM 1913). However, if the first meiotic spindle failed to rotate and the first division were completed while the spindle still paralleled the egg's surface, the second division spindles of the two secondary oocytes may then be far enough apart to form on two separate axes (as suggested for Bombyx by EMERSON 1954).
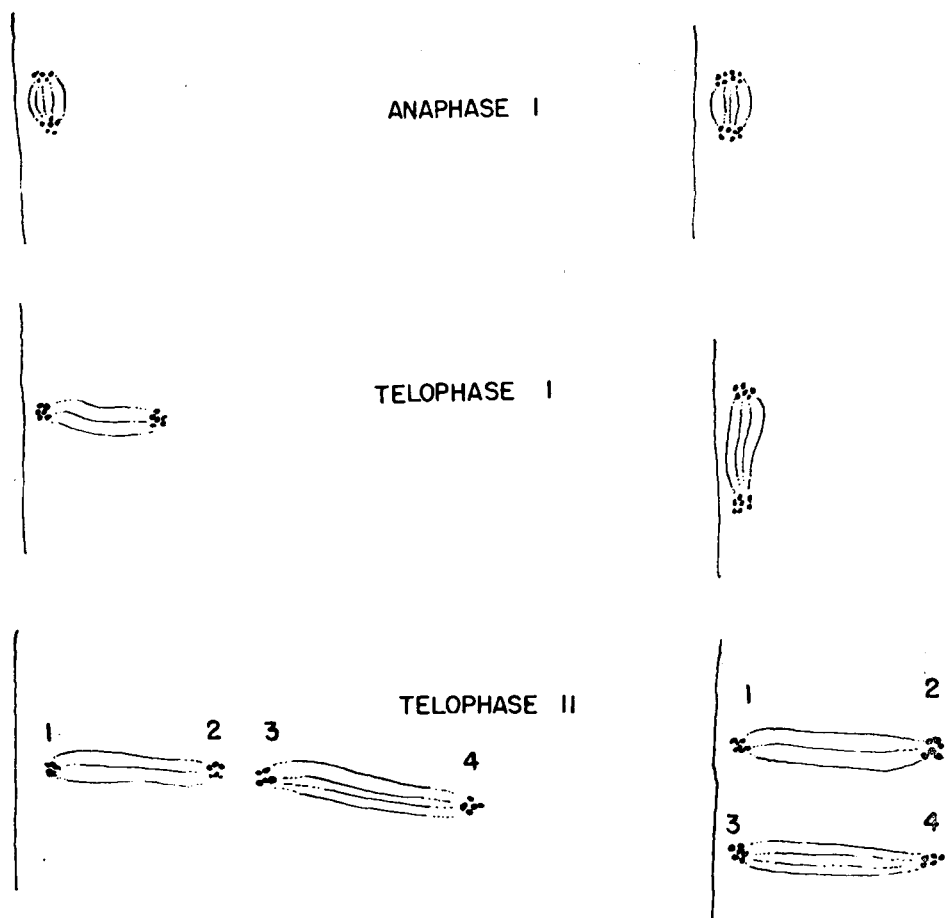
FIGURE 2.—Comparison of hypothetical misorientation scheme (right) with normal meiosis (left).

Two nuclei, 1 and 3, would remain at the periphery of the egg where they would eventually disintegrate. The other two nuclei, 2 and 4, would move into the yolk where they would later unite to form a diploid cleavage nucleus.

The spindle misorientation scheme seems the most likely cytological mechanism to explain the origin of automictic workers. The genetic expectancies of this scheme are identical to those of central union and conform to the observed segregation of mutant genes. This explanation is further favored by the coincidence that automictic eggs probably undergo abnormal treatment during meiosis I, as will be seen below.

Impaternate mosaics

Five gynandromorphs and three mosaic males were found in this investigation. As far as I know, these are the first such bees reported from unfertilized eggs.

Gynandromorphs: The gynandromorphs exhibited variable proportions of male and female tissues in their external and internal morphology. Two of them had very little female tissue and were otherwise similar to the mosaic males. The female parts were either queen or worker in different bees.

Assuming that the origin of the female tissues is the same as the origin of the automictic workers and that the male tissues are haploid, the phenotypes of mutant gynandromorphs suggest that they are 2N-N-N mosaics and that the female and male tissues originate from the same first cleavage nuclei. The phenotypes of two gynandromorphs with only male eye facets indicate that the male tissues arise from two different haploid nuclei: one such bee from an i/i^+ queen had one ivory and one normal compound eye, and another from a ch^z/ch^r queen had streaks and patches of red and chartreuse in both male eyes. The common origin of male and female tissues in the gynandromorphs is illustrated by a single bee from a ch^z/ch^+ queen. This bee, the only one found with mutant phenotypes in tissues of both sexes, exhibited the chartreuse phenotype in its male compound eye as well as in its female compound eye.

These results suggest that two haploid egg pronuclei produced by the misorientation scheme divide at least once before union. Two haploid nuclei, one descending from each of the two secondary oocytes, unite to form a diploid cleavage nucleus which develops into female tissue. The other haploid nuclei develop into mosaic male tissues.

Mosaic males: Three drones found in the progenies of i/i^+ queens and a ch^z/ch^r queen had different eye color phenotypes in each of their two compound eyes corresponding to the two alleles of their heterozygous mothers; hence they appeared to be N-N mosaics. Otherwise they seemed to be normal drones.

The close resemblance of the mosaic males to some of the gynandromorphs suggests that the former developed by the same means as the male tissues in the gynandromorphs.

The proposed origin of the mosaic males from "central nuclei" rather than from "terminal nuclei" is supported by the occurrence of more mosaics from i/i^+ queens than from ch^z/ch^r queens. Bees with mosaic male eyes would be the expression of unlike alleles in each of the two "central nuclei." If segregation in mosaic males were consistent with that of the workers, (Table 2) i-i^+ mosaics would be expected to be more frequent than ch^z-ch^r mosaics. Of the five bees with mosaic male eyes (two gynandromorphs and three drones), three were i-i^+ and two were ch^z-ch^r. However, there were twice as many workers from ch^z/ch^r queens as there were from i/i^+ queens (Table 2). If rates of mosaic male production were equal in the two genotypes, the relative numbers of male mosaics might then be changed to $6\ i$-i^+: $2\ ch^z$-ch^r.

Although the proposed origin of mosaic males is based on only a few bees, the close resemblance of these bees to the gynandromorphs, which in turn seem related to the workers, favors this explanation over alternative proposals.

DISCUSSION

The foregoing analysis suggests that the workers, gynandromorphs, and mosaic males found in this investigation were produced by automictic parthenogenesis in which unusual meiosis leads to the formation of two haploid egg pronuclei, from which these unusual bees develop. No indication of apomictic parthenogenesis was detected.

Furthermore, it seems as though the automictic female tissues are diploid and the mosaic male tissues are haploid, as judged by morphological comparisons to ordinary bees. Also, the observed segregation of mutant genes in these bees is consistent with the assumption of female diploidy and male haploidy. No indications of polyploid origin or of male diploidy were found.

It was assumed that there was no selective mortality of any of the automictic bees. Probably more mortality occurred than was detected, but there is no reason to believe it was selective. At our present state of knowledge (KERR and LAIDLAW 1956) the only known source of selective mortality could be linkage of a gene locus to the lethal alleles. Selective mortality from this cause seems absent with the chartreuse locus and was experimentally shown to be absent with the ivory locus.

The assumption that the centromere divides reductionally in meiosis I is consistent with the most likely explanation of automictic parthenogenesis. Actually this assumption is demonstrated *only* for the chromosome bearing the ivory locus. One should be cautious about generalizing from these genetic data, however, because very few cytological observations demanding only reductional division of the centromere in meiosis I have been made (e.g., prophase interlocking, DARLINGTON 1937, pp. 255–258). On the other hand, other observations indicate either reductional or equational division of the centromere (MATSUURA 1939).

Additional support for the proposed origin of automictic bees by the spindle misorientation scheme would be the fulfillment of the expectation of producing certain mosaics from *fertilized* eggs.

TABLE 3

Types of mosaics expected with automictic development in combination with fertilization

	No cleavage before fertilization	Cleavage before fertilization	
		No union of maternal nuclei	Union of maternal nuclei
	A. One nucleus fertilized		
Karyotype*	$2N_b - N_m$	$2N_b - N_m - N_m$	$2N_b - 2N_m - N_m$ and $2N_b - 2N_m - N_m - N_m$
	B. Two nuclei fertilized		
Karyotype*	$2N_b - 2N_b$	$2N_b - 2N_b - N_m - N_m$	$2N_b - 2N_b - 2N_m$ and $2N_b - 2N_b - 2N_m - N_m - N_m$

* $2N =$ diploid; $N =$ haploid; $_b =$ biparental; $_m =$ maternal.

The number of different types of mosaicism expected (Table 3) depends on whether one or both of the egg pronuclei are fertilized, whether the egg pronuclei could divide before fertilization occurs, and (if division before fertilization is possible) whether union of maternal nuclei could coexist with fertilization. Fertilization after cleavage seems inconsistent with the sequence of events in normal fertilized eggs, since the male and female pronuclei unite before cleavage and all extra male pronuclei form irregular division figures *simultaneously* with the first cleavage division of the zygote and disintegrate shortly after the second cleavage division (NACHTSHEIM 1913). If extra male pronuclei behave the same as in normal fertilized eggs, the mosaic types requiring fertilization after cleavage seem less likely than those requiring fertilization before it. Perhaps single fertilizations may be favored over double fertilizations if repulsion between male pronuclei (NACHTSHEIM 1913) is strong enough.

On the basis of these considerations, gynandromorphs with biparental female tissues and maternal male tissues ($2N_b–N_m$) might be expected to be most frequent, mosaic females from two biparental zygotes ($2N_b–2N_b$) next frequent, and various other mosaics listed in Table 3 least frequent or entirely absent. It is of interest that BOVERI's (1915) hypothesis for gynandromorph formation, the fertilization of one of the two second cleavage nuclei and the haploid development of the other, would be classed with the *least likely*. If gynandromorphs could be formed by BOVERI's scheme, several sorts of gynandromorphs, distinguished by mosaic male tissues, could also be formed. The phenotype expected from BOVERI's hypothesis would be identical to that of the $2N_b–N_m$ gynandromorph expected in the present scheme.

To date, two types of mosaics listed in Table 3 have been found: a $2N_b–N_m$ gynandromorph (MACKENSEN 1951) and 14 $2N_b–2N_b$ mosaic females (TABER 1955). Although further investigation of fertilized eggs is necessary, it seems probable that single and double fertilization, respectively, of binucleate eggs formed by the misorientation scheme can account for these mosaic bees.

The genetic segregation in the automictic workers (Table 2) provides a basis for mapping the distance between a gene locus and its centromere. Assuming random recombination in "central union," 50 percent of the equational divisions in meiosis I result in homozygosis in the diploid nucleus. Thus equational divisions in meiosis I should be four times as frequent as homozygous recessive workers. Therefore, estimates of recombination between gene locus and centromere are 7.2 for ivory and 57.5 for chartreuse (ch^s/ch^r and ch^s/ch^+ progenies averaged). The map distances between gene locus and centromere are 3.6 units for ivory and 28.8 units for chartreuse. These estimates must be considered tentative, however. Ivory could be overestimated since it is based on a single i/i worker. Chartreuse, on the other hand, could be underestimated since an excess of equational divisions could indicate a distance of more than two chiasmata between the centromere and gene locus (MATHER 1935).

As a system of mating, automixis could produce variable results, depending on the linkage of the genes to their respective centromeres. Heterozygosity would

tend to be retained in very closely linked genes. A gradual decrease in the amount of retained heterozygosity would be expected for gene loci which are progressively less closely linked. If in honey bee chromosomes there is a possibility for three or four crossovers to occur between a gene and its centromere, equational division should theoretically approach 66.6 percent (MATHER 1935). At this level the loss of heterozygosity would reach its maximum, 33.3 percent per generation, for this "mating" system.

As compared with other mating systems, the rate of inbreeding per generation for loosely linked genes would be somewhat less than that for full sister mating (POLHEMUS and PARK 1951). On a time basis, automixis would be more efficient for loosely linked genes than full sister mating, because twelve generations could be produced each year as compared to about five generations per year with full sister mating. In one year it would be possible to eliminate 99 percent of the initial heterozygosity from the genes that are loosely linked to their centromeres. However, genes at the level of the ivory locus can lose only 3.6 percent of their heterozygosity per generation and only 35 percent in 12 generations; it would take 67 generations (5½ years) to eliminate 90 percent of the initial heterozygosity.

Frequency of occurrence of automictic workers

Automictic workers did not occur in a chronologically constant proportion. Estimates of the percentage of workers decreased with increased numbers of progeny from individual unmated queens (0.19 percent with 13,172 bees from 18 queens; 0.09 percent with 81,620 bees from 16 queens).

Most workers occurred among a queen's initial brood, where they constituted from less than one percent to as much as seven percent of the total progeny. Of 14 unmated queens which produced brood for two to three months and which produced workers, ten (71 percent) produced most of their workers in the first 15 days of laying. Also, 21 sister queens, producing brood for an average of only five days, averaged 4.4 workers per queen (range: 0–13), nearly equalling the average of 5.3 workers per queen (range: 2–7) produced by four other sisters of these queens over a period of two to three months.

Few, if any, workers occurred in later brood, and these "late" workers often occurred during acceleratory periods of fluctuating brood production.

The pattern of worker distribution suggests that both "late" and "early" workers originate during accelerating oviposition following an absence or low rate of oviposition. Further experiments confirmed this suggestion. Eleven queens were overwintered and permitted to produce brood in a second season (Table 4). In the first brood of the season, one worker was produced, as expected, by a queen (I-1) which started laying more heavily than the other queens which started laying very gradually. In later brood before the cage tests, one worker was initiated two days after the alleviation of restricted oviposition; otherwise workers were not expected. The same queens were caged to interrupt laying, then released into very strong nuclei to induce laying again. In the first cage test,

TABLE 4

Chronology of production of automictic workers by overwintered unmated queens

Queen number	Date first laying	Workers produced 1953	First brood of season	Workers, 1954 Later brood before cage tests	First cage test	Second cage test
G–1	5– 6–53	6	0	0	3	0
G–3	5–13–53	2	0	0	0	0
G–4	5–18–53	7	0	0	–‡	–
H–8	5–30–53	37	0	0	–‡	–
J–1	2– 5–54	–*	0	0	3	–§
J–2	2– 8–54	–*	0	0	0	–§
J–3	9–27–53	0†	0	0	2	–§
J–4	9–27–53	1	0	0	0	–§
I–1	10– 9–53	0	1	1	0	0
I–2	10– 7–53	0	0	0	0	0
I–3	9–27–53	0†	0	0	0	0

* No brood produced.
† Brood given poor care by bees in feeder colony.
‡ Queen killed.
§ Queen used for another purpose.

one queen (G-1) produced as many workers in five days as she had in her first 15 days of laying ten months earlier. More workers might have been produced in the first cage test if all the queens had stopped laying while they were caged. The queens may not have been adequately tested in the second cage test, since they laid for only three or four days. Seven younger unmated queens were permitted to start laying for a second time. They had produced their initial brood one month previously and had been caged in a nursery colony in the interim. All the queens which produced sufficient brood produced at least one worker in the second laying period (Table 5).

The chronological distribution of automictic workers is similar to that of irregu·· larities in *Drosophila melanogaster* for which HANNAH (1955a, 1955b) concluded that aged eggs were responsible. Although different cytological phenomena are responsible, both cases represent cytological accidents. HANNAH (1955a) proposed that aged eggs present a sum of environmental conditions which favor cytological accidents.

TABLE 5

Production of automictic workers by new unmated queens during two ovipositional periods

Queen number*	Initial laying (average 5 days)	Laying after caging in nursery (average 3 days)
G– 6	9	1
G– 8	9	6.
G–10	3	3
G–12	11	3

* In addition two queens were lost during the second ovipositional period and another queen produced insufficient brood.

In the present study, eggs could have been aged by unusually long retention in the queen's ovaries. Usually eight or nine days elapsed between carbon dioxide anaesthetization, when the oldest ovarial eggs should have been nearly mature (FYG 1952), and initial oviposition. This time period could have provided a chance for the oldest initial eggs to become aged. After an artificially induced cessation, laying was resumed in so short a time, often within 24 hours, as to suggest that some eggs might have been retained from the previous ovipositional period.

Thus aged eggs might occur in unmated queens immediately preceding ovipositional periods during which automictic bees were known to be initiated.

If mature eggs were retained within the queen before oviposition, nuclear activity would be suspended during meiosis I before spindle reorientation since the oldest ovarial eggs in a laying queen are in meiosis I (PETRUNKEWITSCH 1901), which is not completed until after the egg is laid (PETRUNKEWITSCH 1901; NACHTSHEIM 1912, 1913).

This analysis further supports the conclusion indicated by the genetic analysis: that automictic workers arise from eggs in which a cytological accident had occurred in meiosis I.

In the experimental production of gynandromorphs by chilling newly-laid fertilized eggs (RÖSCH 1927; JORDAN 1952), the cold might have an effect parallel to the aging of unfertilized eggs. Nuclear development could also be affected during anaphase of meiosis I before spindle reorientation, and similar results might be expected. Further investigation of the effect of chilling newly-laid eggs, both fertilized and unfertilized, should provide decisive information.

Impaternate workers were no more prevalent in brood from laying workers than in brood from unmated queens. Six out of eight laying worker colonies produced one or more workers. The chronology of occurrence of their workers is difficult to interpret, because several (to many) individual laying workers produce the progeny in any one colony.

Comparative automictic production by unmated queens of different lines

The comparison of automictic worker production by different lines is somewhat confounded by the relationship of automictic worker production to ovipositional activity. Perhaps the best estimates are the number of workers per queen and the proportion of queens which produced workers (Table 6). These estimates suffer from a lack of standard conditions during the production periods: nucleus strength differed, and some queens had greater opportunity to produce workers in later brood than did others. Nevertheless, real differences seemed to exist, at least between some of the different lines (e.g., lines F and G) during periods of time when the unmated queens had approximately equal maintenance.

Despite the quantitative and qualitative limitations of the data, automictic workers were produced in all lines of every race tested. Differences between lines were as great as differences between races.

The data are inadequate for generalizations about the occurrence of automictic parthenogenesis in various racial types. Such generalizations should be

withheld until a more precise means of measuring automictic reproduction is available and thorough investigations are made of the racial types throughout the world.

CONCLUSIONS

The foregoing analysis of the genetic segregation in, and frequency of automictic workers suggests the following sequence of events to account for automictic workers, gynandromorphs, and mosaic males. Before initial oviposition or during a later inhibition of oviposition a mature unfertilized egg is retained within the queen for an unusually long time. During this time meiosis is suspended in anaphase I. The normal reorientation of the first division spindle is inhibited by this "aging," so that after the egg is laid meiosis II occurs with the two second division spindles on two separate axes. Two polar bodies and two egg pronuclei are formed. The polar bodies take no further part in development. In most of the unusual eggs, the two egg pronuclei unite to form a diploid cleavage nucleus which develops into a female. Rarely, the two egg pronuclei develop separately as two haploid cleavage nuclei to form a mosaic male. Two unlike haploid cleavage nuclei, one descending from each of the two secondary oocytes

TABLE 6

Production of automictic workers by unmated queens of different honey bee lines

Racial type and line*	Number of queens tested	Percentage of queens producing workers	Number of workers produced		
			Total	Range per queen	Average per queen
European brown					
A	3	33.3	3	0– 3	1.00
Caucasian					
B	5	100.0	9	1– 3	1.80
C	5	60.0	6	0– 4	1.20
D	2	100.0	3	1– 2	1.50
Total	12	83.3	18		1.50
Yellow Italian					
E	3	66.7	4	0– 2	1.33
G	25	92.0	130	0–13	5.20
Total	28	89.3	134		4.78
Leather-colored Italian					
F	5	60.0	5	0– 3	1.00
H	8	87.5	49	0–37	6.13
I†	3	33.3	2	0– 2	0.67
J†	4	75.0	6	0– 3	1.50
Total	20	70.0	62		3.10
TOTAL	63	79.4	217		3.44

* All queens in each line were F, daughters of a single parent queen.
† Queens in line I were daughters of a cross between G and H lines; queens in line J were daughters of a cross between G and F lines.

after at least one cleavage division, unite to form a diploid cleavage nucleus, which develops together with the remainder of the haploid cleavage nuclei to produce a gynandromorph with mosaic male tissues.

SUMMARY

1. Unfertilized eggs of unmated queens and laying workers gave rise to a small proportion of workers and, more rarely, to gynandromorphs with mosaic male tissues and to mosaic males. The females tissues were identical to worker and queen tissues of zygogenetic origin and the male tissues were identical to normal drone tissues. Hence the female tissues were probably diploid and the male tissues haploid.

2. The proportion of workers was highest (usually one percent or less, but as high as seven percent) during accelerated oviposition just after laying began or following an artificially induced cessation of oviposition. Few workers were produced during sustained oviposition, and these were often associated with accelerations following inhibitions in fluctuating oviposition. This pattern of the occurrence of the workers suggests that they are derived from eggs which are retained in the ovaries of the queens for unusual lengths of time before oviposition.

3. Unmated queens heterozygous for mutant genes produced homozygous recessive workers: 12.4 and 19.6 percent with chartreuse (ch^z) and 1.8 percent with ivory (i). The low proportion of i/i workers was probably due to linkage of this gene locus to its centromere and was not caused by selective mortality.

4. It is concluded that the automictic workers are derived from the union of two haploid nuclei formed by complete meiosis. Each of the two nuclei involved in union are from different secondary oocytes. The following cytological scheme is proposed. Normal orientation of the first meiotic spindle is interfered with during unusual retention of mature eggs within the queen, so that the second division spindles form on two different axes. Completion of this unusual second division results in the formation of two haploid egg pronuclei and two haploid polar bodies. Union of the two egg pronuclei forms a diploid cleavage nucleus which develops into a worker. The gynandromorphs and mosaic males can be traced to similar origin. Cleavage of the two haploid egg pronuclei form mosaic male tissues, and the union of two haploid nuclei descending from each of the two secondary oocytes forms a diploid cleavage nucleus from which the female tissues of the gynandromorphs are derived.

5. The chartreuse gene (ch^z) segregates as though it were loosely linked to its centromere at an estimated distance of 28.8 units. The ivory gene (i) segregates as though it were linked closely to its centromere at an estimated 3.6 units. Segregation of the ivory gene is independent of the lethal alleles.

6. Automixis should produce variable results as a system of "mating," depending on the linkage of a gene to its centromere. Theoretically, loss of heterozygosity per generation should be lowest, 0.0 percent, with complete linkage, and should reach its highest efficiency, 33.3 percent, with loose linkage.

7. Automictic workers were found in all races tested: one line of European Brown, three lines of Caucasian, four lines of Leather-colored Italian, and two lines of Yellow Italian. Differences between lines were as large as or larger than differences between races. Unmated queens of most lines produced only a few workers, but one exceptional producer (37 workers) occurred in one of the Leather-colored Italian lines, and several exceptional producers (5 to 13 workers) were found in a Yellow Italian line.

LITERATURE CITED

ANDERSON, J., 1918 A letter from Scotland (to C. P. DADANT). Am. Bee J. **58**: 308.

BALDENSPERGER, P. J., 1918 Punics and parthenogenesis. Am. Bee J. **58**: 375–376.

BOVERI, TH., 1915 Über die Entstehung der Eugsterschen Zwitterbienen. Arch. Entwicklungsmech. Organ. **41**: 264–311.

BUTLER, C. G., 1954 *The World of the Honeybee*. Collins. London.

CATCHESIDE, D. G., 1951 *The Genetics of Microorganisms*. Pitman and Sons. London.

DADANT, C. P., 1919 Punic or African bees and parthenogenesis. Am. Bee J. **59**: 419–420.

DARLINGTON, C. D., 1937 *Recent Advances in Cytology*. Blakiston. Philadelphia.

EMERSON, S., 1954 Frequencies of pre- and post-reduction of the sex chromosomes of *Bombyx mori* based on GOLDSCHMIDT and KATSUKI's studies of gynandromorphs and mosaics. Cytologia **19**: 144–151.

FYG, W., 1949 Können bei der Honigbiene Arbeiterinnen und Königinnen auch aus unbefruchteten Eiern entstehen? Schweiz. Bienen-Zeit., N. F. **72**: 564–570; also 1950 Bee World **31**: 17–19; also 1952 Gaz. Apicole **53**: 206–209.
    1952 The process of natural mating in the honeybee. Bee World **33**: 129–139.

GUBIN, A. F., and I. A. KHALIFMAN, 1951 (Queen rearing from drone eggs.) Agrobiologiya **1**: 99–104. (In Russian.)

HANNAH, A., 1955a The effect of aging the maternal parent upon the sex ratio in *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **86**: 575–599.
    1955b Environmental factors affecting elimination of the ring-X chromosome in *Drosophila melanogaster*. Z. Ind. Abst. Vererb. **86**: 600–621.

HEWITT, J. (HALLAMSHIRE BEEKEEPER), 1892 Fertile workers—their utility. J. Hort. London **25**: 134.

JACK, R. W., 1917 Parthenogenesis amongst the workers of the Cape honeybee: MR. G. W. ONIONS' experiments. Trans. Roy. Ent. Soc. London **1916**: 396–403.

JORDAN, R., 1952 Interessante Folgen von Brutverkühlungen. Österreich. Imker **2**: 28–30.

KERR, W. E., and H. H. LAIDLAW, JR., 1956 General genetics of bees. Advances in Genet. **8**: 109–153.

LAIDLAW, H. H., JR., 1954 Beekeeping management for the bee breeder. Am. Bee J. **94**: 92–95.

LAIDLAW, H. H., JR., and J. E. ECKERT, 1950   *Queen Rearing.* Dadant and Sons. Hamilton, Illinois.

LAIDLAW, H. H. JR., M. M. GREEN, and W. E. KERR, 1953   Genetics of several eye-color mutants in the honey bee. J. Heredity **44**: 246–250.

MACKENSEN, O., 1943   The occurrence of parthenogenetic females in some strains of honeybees. J. Econ. Entomol. **36**: 465–467.

   1947   Effect of carbon dioxide on initial oviposition of artificially inseminated and virgin queen bees. J. Econ. Entomol. **40**: 344–349.

   1951   Viability and sex determination in the honeybee (*Apis mellifera* L.). Genetics **36**: 500–509.

MATHER. K., 1935   Reductional and equational separation of chromosomes in bivalents and multivalents. J. Genet. **30**: 53–78.

MATSUURA, H., 1939   Chromosome studies on *Trillium kamtschaticum* Pall. VII. Additional evidence for the neo-two-plane theory of bivalent constitution. Cytologia **9**: 78–87.

NACHTSHEIM, H., 1912   Parthenogenese, Eireifung, und Geschlechtsbestimmung bei der Honigbiene. Sitzber. Ges. Morph. Physiol. München **28**: 22–29.

   1913   Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica* L.). Arch. Zellforsch. **11**: 169–241.

ONIONS, G. W., 1912   South African "fertile worker bees." Agric. J. Union S. Africa **3**: 720–728.

   1914   South African "fertile" worker bees. Agric. J. Union S. Africa **7**: 44–46.

PETRUNKEWITSCH, A., 1901   Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. Zool. Jahrb., Abt. Anat. u. Ontog. Tiere **14**: 573–608.

POLHEMUS, M. S., and O. W. PARK, 1951   Time factors in mating systems for honey bees. J. Econ. Entomol. **44**: 639–642.

RÖSCH, G. A., 1927   Über einen Weg, Zwitter der Honigbiene (*Apis mellifica* L.) im Experiment zu erzeugen. Sitzungsber. Ges. Morph. Physiol. München **37**: 71–81.

ROTHENBUHLER, W. C., J. W. GOWAN, and O. W. PARK, 1953   Five mutant genes in honey bees (*Apis mellifera* L.). J. Heredity **44**: 251–253.

SPEICHER, K. G., 1934   Impaternate females in Habrobracon. Biol. Bull. **67**: 277–293.

SPEICHER, K. G., and B. R. SPEICHER, 1938   Diploids from unfertilized eggs in Habrobracon. Biol. Bull. **74**: 247–252.

STALKER, H. D., 1954   Parthenogenesis in Drosophila. Genetics **39**: 4–34.

SUOMALAINEN, E., 1950   Parthenogenesis in animals. Advances in Genet. **3**: 193–253.

TABER, S., III, 1955   Evidence of binucleate eggs in the honey bee. J. Heredity **46**: 156.

WARMELO, D. S. VAN, 1912   South African fertile-worker bees and parthenogenesis. Agric. J. Union S. Africa **3**: 786–789.