Isogenic sperm line maintenance in the honey bee

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IN THE HONEY BEE, *Apis mellifera* L., polyspermy is the usual case[12]. One sperm pronucleus unites with the egg pronucleus to form the zygote, and the other (accessory) sperm pronuclei degenerate[12]. On rare occasions mocaics arise as a result of the development of an accessory sperm pronucleus[15], the union of an accessory sperm pronucleus with an additional egg pronucleus to form a second zygote[18], or the union of two sperm pronuclei to form a second diploid nucleus[8]. Rothenbuhler et al.[17] investigated a stock of bees that frequently produces gynandromorphic progeny containing discrete patches of female and male tissue. They showed that the female tissue arose from a biparental zygote and the male tissue from the development of an accessory sperm pronucleus (zygogenesis and androgenesis)[15]. For a review of these and other aspects of bee genetics, see Kerr and Laidlaw[5], and Rothenbuhler et al.[16].

A normal honey bee drone (male) develops from an unfertilized egg[3]. He is haploid in origin and through abortive meiosis produces several million sperm cells that are genetically the same as the egg from which he developed[12]. Similarly, Rothenbuhler et al.[15] have shown that a functionally male gynandromorph produces semen containing sperm cells that are genetically the same as the accessory sperm from which the gynandromorph's male tissue developed.

Tucker and Laidlaw[19] hypothesized that there is a potential to multiply an isogenic sperm line by using a stock that produces gynandromorphs with androgenic male tissue. The procedure would involve successive matings of gynandromorph-producing queens with semen from gynandromorphs.

Isogenic sperm lines would have application in the production of bees to be used as experimental units and in bee

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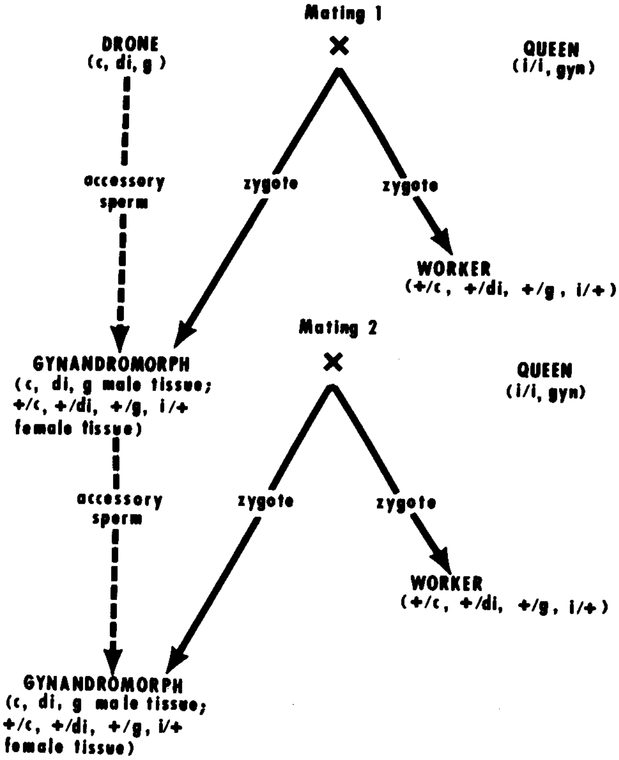

FIGURE 1—The mating scheme for isogenic sperm line maintenance in the honey bee. The broken line represents a genetically marked sperm line that was maintained for two generations of propagation by androgenesis.

breeding. For studies involving characteristics of the entire honey bee colony, it can be important to have colonies of very similar individuals[13] or very similar colonies of heterogeneous individuals. The former can be achieved by the mating of highly inbred queens to an isogenic sperm line, the latter by the mating of hybrid queens (from the cross of two highly inbred lines) to an isogenic sperm line. For studies involving characteristics of individual bees, a reduction in the variance among progeny of different queens can be achieved by mating those queens to an isogenic sperm line[6]. As a breeding tool, an isogenic sperm line serves to sustain an individual gametic genome for programs involving gametic selection or backcrosses to a gamete (for rapid inbreeding[1] or reconstitution of a line from stored semen[4]). The following experiment tests the hypothesis that an isogenic sperm line can be maintained by androgenesis.

Materials and Methods

For this experiment it was necessary to be able to genetically identify sperm lines and detect any introduction of maternal genome into the sperm lines. In order to identify sperm lines a multi-marked stock was developed so that drones could be obtained that were hemizygous for the mutant genes cordovan body color (*c*)[9], diminutive wing (*di*)[20], and garnet eye color (*g*)[7]. The maternal genome was genetically marked by using only homozygous ivory-eyed (*i/i*)[17] queens of the gynandromorph-producing stock.

Several isogenic sperm lines were initiated. In each case, one multiple-marked drone was mated[10] to one gynandromorph-producing queen (Figure 1, mating 1). Each queen was maintained in a small colony until it could be determined if her progeny included gynandromorphs.

Queens producing gynandromorphs were transferred into full-size colonies and confined to the brood nest by queen excluders (sheets of material with spaces of a size that permit the passage of worker bees but not drones or queens). The colonies were fed sugar syrup to stimulate brood production. Six weeks after the queens began to lay in the full-size colonies, the queen excluders were set ajar, and the escaping gynandromorphs were collected. The gynandromorphic progeny of each queen was collected and kept separate.

Semen was collected from the gynandromorphs (all semen from one queen's progeny was pooled), and collections from different queens (different sperm lines) were kept separate. The semen was used to inseminate a second group of gynandromorph-producing queens (Figure 1, mating 2).

Second-group queens were maintained in full-size colonies. Each queen's gynandromorphic and worker (female) progenies were scored for phenotype.

Results

Of 57 single-drone-inseminated queens, 43 survived to the egg-laying stage. Forty-two of these laid fertilized eggs that developed into wild-type workers or gynandromorphs with wild-type female tissue and cordovan, diminutive, garnet male tissue. One queen laid unfertilized eggs that developed into ivory-eyed drones. Four queens produced a sufficient proportion of gynandromorphs to be eligible for transfer into full-size colonies.

Two of the four eligible queens were transferred into full-size colonies. The gynandromorphic progeny from one of the queens produced 8 μl of semen that was used to inseminate four gynandromorph-producing queens. The gynandromorphic progeny of the other queen produced 4 μl of semen that was used to inseminate two gynandromorph-producing queens. Approximately 50 percent of the gynandromorphs yielded ⅓ to ½ μl of clean semen. The rest were sterile or had feces in their genital tract.

Of the six queens inseminated with semen from gynandromorphs, one died, four produced only wild-type worker progeny, and one produced both wild-type worker progeny and gynandromorphs with wild-type female tissue and cordovan, diminutive, garnet male tissue.

Discussion

The presence of cordovan, diminutive, garnet male tissue in the gynandromorphs produced by the second-group queen demonstrates that the genome of the original drone's semen remained intact through two generations of propagation by androgenesis. This confirms the androgenic origin of male tissue in gynandromorphs that are the progeny of functionally-male gynandromorphs. Semen from single drones (approximately 1 μl) was used to develop a sperm line represented by 8 μl of semen in one case and 4 μl of semen in another. Thus the possibility of maintaining and increasing an isogenic sperm line is confirmed. The absence of ivory-eyed workers in the same progeny indicates that maternal genes do not contaminate the sperm line being propagated.

The procedure developed for producing and screening gynandromorphs for a known gynandromorph-producing queen proved satisfactory. In the full-size colony the queen's egg-laying rate was increased and consequently the proportion[2] and total number of gynandromorphs was increased. The gynandromorphs with male (larger) thoraces were confined to the brood nest by the excluder; these were also likely to have male abdomens[11] and hence, male genetalia. Thus, the most desirable gynandromorphs were confined to the area where worker bees continued to feed them optimally.

For isogenic sperm line maintenance to be employed with a predictable and acceptable degree of efficiency, the gynandromorph-producing stock would have to be bred for increased frequency of gynandromorph-producing queens. In this study less than 11 percent (5/46) of the queens in the gynandromorph-producing stock were gynandromorph producers. When the stock was being maintained under selection, 5 percent to 40 percent of the progeny of most queens was gynandromorphic[14]. Laidlaw (private communication) was able to select for similar levels of gynandromorph production in an unrelated stock. If selection were again imposed on the gynandromorph-producing stock, and the previous levels of gynandromorph production achieved, the technique of isogenic sperm line maintenance could become a practical bee-breeding tool.

Summary

Isogenic sperm lines in the honey bee (*Apis mellifera* L.) were maintained for two generations of propagation by androgenesis. The androgenic origin of male tissue in gynandromorphs that are the progeny of functionally male gynandromorphs was confirmed. This was demonstrated using three genetic markers (cordovan, diminutive, and garnet) in the sperm line and one genetic marker (ivory) in a gynandromorph-producing stock. A procedure is described for the initiation of a sperm line by using a single drone's semen to inseminate a gynandromorph-producing queen, and propagation of the sperm line by inseminating gynandromorph-producing queens with semen from gynandromorphs with androgenic male tissue.

Literature Cited

1. CROW, J.F. and W.C. ROBERTS. Inbreeding and homozygosis in bees. *Genetics* 35:612-621. 1950.
2. DRESHER, W. Der Einfluss von Umweltbedingungen auf die Bilding von Gynandromorphen bei der Honigbiene *Apis mellifica* L. *Insectes Soc.* 12:201-218. 1965.
3. DZIERZON, J. Gutachten über die von Hrn. Direktor Stöhr im ersten und Zweiten Kapitel des General-Gutachtens aufgestellten Fragen. *Bienen-Ztg* (Eichstätt) 1:109-113; 119-121. 1845.
4. HARBO, J.R. Storage of honeybee spermatozoa at −196° C. *J. Apic. Res.* 18:57-63. 1979.

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

6. KUBASEK, K.J. Isogenic sperm lines in the honey bee (*Apis mellifera* L.). M.S. thesis, Louisiana State Univ., Baton Rouge, 1977.

7. LAIDLAW, H.H. JR., M.A. EL BANBY, and K.W. TUCKER. Five new eye-color mutants in the honey bee. *J. Hered.* 55:207–210. 1964.

8. ——— and K.W. TUCKER. Diploid tissue derived from accessory sperm in the honey bee. *Genetics* 50:1439-1442. 1964.

9. MACKENSEN, O. Viability and sex determination in the honey bee (*Apis mellifica* L.). *Genetics* 36:500–509. 1951.

10. ——— and K.W. TUCKER. Instrumental Insemination of Queen Bees. *U.S.D.A. Handbook* 390. 1970.

11. MILNE, C.P. Morphogenic fate map of prospective adult structures of the honey bee. *Dev. Biol.* 48:473–476. 1976.

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

13. ROTHENBUHLER, W.C. A technique for studying genetics of colony behavior in honey bees. *Am. Bee J.* 100:176–178. 1960.

14. ———. Progress and problems in the analyses of gynandromorphic honey bees. *Proc. Xth Int. Cong. Entomol.* 2:867–874. 1956(1958).

15. ———, J.W. GOWEN, and O.W. PARK. Androgenesis with zygogenesis in gynandromorphic honeybees (*Apis mellifera* L.). *Science* 115:637–638. 1952.

16. ———, J.M. KULINČEVIĆ, and W.E. KERR. Bee genetics. *Ann. Rev. Genet.* 2:413–438. 1968.

17. ———, M.S. POLHEMUS, J.W. GOWEN, and O.W. PARK. Gynandromorphic honey bees. *J. Hered.* 40:308–311. 1949.

18. TABER, III, S. Evidence of binucleate eggs in the honey bee. *J. Hered.* 44:156. 1955.

19. TUCKER, K.W. and H.H. LAIDLAW, JR. The potential for multiplying a clone of honey bee sperm by androgenesis. *J. Hered.* 57:213–214. 1966.

20. WITHERELL, P.C. and H.H. LAIDLAW, JR. Behavior of the honey bee (*Apis mellifera* L.) mutant, diminutive-wing. *Hilgardia* 45:1–30. 1977.