AUTOMATIC FREQUENCY RESPONSE IN THE EVOLUTION OF MALE HAPLOIDY AND OTHER COCCID CHROMOSOME SYSTEMS[1]

SPENCER W. BROWN

Department of Genetics, University of California, Berkeley

Received December 23, 1963

IT can be shown that a gene responsible for a certain change in the life cycle of an organism will itself be automatically increased or decreased in frequency, with selection and random fluctuations remaining inconsequental. This phenomenon has been called automatic frequency response (AFR), and applied in a partial explanation of the sequence or order in which certain chromosome systems appear to have evolved in the coccids (Homoptera : Coccoidea) (BROWN 1963). The purpose of the present report is to develop the concept in more detail, especially in regard to male haploidy, and to provide models demonstrating the rate at which one allele will be replaced by another. In addition, a quite unexpected aspect of the evolution of male haploidy has since become evident: haploid males will tend to replace diploid males without benefit of further genetic change. Certain, but not all, of the changes yielding an AFR are similar to those resulting in meiotic drive as defined by SANDLER and NOVITSKI (1957). These two processes will be compared in the discussion after examples of AFR have been described and the role of selection and random fluctuations in an AFR system have been considered.

It should also be made clear at this juncture that there are no examples known of an AFR, and the entire treatment will therefore be theoretical. The only confirmation yet discovered is that of the probable order of appearance, during evolution, of certain chromosome systems in the coccids (BROWN and MCKENZIE 1962; BROWN 1963). This evolutionary sequence itself seems, at first glance, to be so highly improbable that an explanation of it would not be without significance. The pertinent characteristic is the type of hereditary transmission from the males in each of the four successive systems: in the basic system, the males transmit as diploids (or sub-diploids since they are X0); in the second, the males transmit as haploids; in the third as sub-diploids; and in the fourth as haploids again. In general terms, the evolutionary sequence thus alternates: diploid, haploid, diploid, haploid.

Male Haploidy

Prerequisite conditions: In the Metazoa, the antecedents of the species or groups with male haploidy have undoubtedly all been diploid (WHITE 1954). In each

[1] Supported in part by Grants G-9772 and GB-413 from the National Science Foundation.

of these cases, the first haploid males to appear would have had to compete with diploid males. If not at least to some extent successful in such competition, either directly or because of an eccentricity in the life cycle, the haploid males would have been of no significance except, perhaps, for nuisance value. There would seemingly be no selective differential between an unfertilized egg, a haploid male dying in the pupal stage, and a vigorous but sterile adult; thus there could have been no selection for male haploidy prior to the appearance of functional males, and the first appearance of such males must have been due therefore to accident or chance.

The cases cannot be reviewed in detail here, but examples are known from the coccids of the production, through failure of a chromosomal mechanism, of a small percentage of variant individuals which may or may not reach maturity (NUR 1963; BROWN and associates, unpublished). Furthermore similar variants are produced by similar, though not directly related species. Thus the accidental occurrence of a successful pioneer of a new type of life cycle may have been the consequence of the production, to varying degrees of perfection, of the variant individuals by a fairly large number of species over a long span of years.

HUGHES-SCHRADER (1948) has listed some of the physiological prerequisites for the appearance of haploid males, such as the removal of the barrier to development without fertilization. As far as genetic prerequisites are concerned, it seems unlikely that male haploidy would appear in a species dependent to any appreciable extent on heterozygosity. To the contrary, species adapted to inbreeding would be much more likely progenitors. The population structure of the coccids is typically colonial, thus favoring inbreeding, and haploid and haploid-like systems have originated independently at least twice in this group. In addition, the sex determining mechanism must be such that the haploid embryos will develop into males. It seems unlikely that an XX-XY mechanism would precede male haploidy except under special circumstances. In the coccids, XX-X0 systems are known to precede both true male haploidy and the haploid-like lecanoid system (HUGHES-SCHRADER 1948). The haploid males under this circumstance would be expected to be XA (A = a haploid set of autosomes), and, barring nondisjunction, all their sperm would be XA and all their offspring therefore female. Since there would have been no prior selection for modifications of spermatogenesis that would preclude the deficient gametes expected from a single haploid set undergoing meiosis by itself, a low chromosome number, frequently giving complete gametes by chance, would certainly have been a favorable preexisting condition. It is therefore of interest to note that in the haploid iceryine coccids n = 2, and in their XXAA-X0AA predecessors A = 2 also (HUGHES-SCHRADER 1948).

It will be shown in the next section that genes responsible for the presence of haploid males will be automatically increased in frequency even in face of considerable adverse selection. The rarity of male haploidy in the animal kingdom may thus follow to a considerable extent from the stringent requirements for its first appearance rather than from subsequent trials. To whatever extent,

however, either AFR or selection may be assumed to be responsible for the establishment of fully fledged male haploidy, the same prerequisites must be assumed for the first appearance of functional haploid males whether or not, of course, these include the ones suggested here.

Automatic frequency response in the evolution of male haploidy: The model used in most of the calculations to be reported here is one in which a colony is founded by two individuals, a heterozygous female and a homozygous or haploid male. It is assumed that the colony reaches substantial size, 100 to 200 pairs, in the first generation thereafter, and is maintained sufficiently large to preclude appreciable effects on gene frequency by fluctuation in population size. (On the other hand, the founding of colonies from only two individuals gives the maximum opportunity for random fixation.) It is further assumed that breeding is at random within the colony.

For the first specific example, it is assumed that each female produces 90 percent fertilized eggs, and 10 percent unfertilized eggs. In females with the gene *H*, the unfertilized eggs undergo development; in those without *H*, they do not. It is furthermore assumed that the haploids may develop poorly, be less vigorous than diploid males, or less fertile; for all of these factors a net of 50 percent has been assumed, that is, two haploid males are required to make the same contribution to the next generation as one diploid male.

The sequence of events during the first few generations is shown in Table 1. The original *Hh* mother will produce half sons and half daughters from the 90 percent fertilized eggs and all sons from the 10 percent unfertilized eggs. The net percent from these 10 percent haploid sons is 5 percent according to the handicap arbitrarily set at 50 percent. Total males then equal 50 percent of which 45 percent are diploid and 5 percent the net haploid; for the haploids the net functional percent of the total functional males is thus 5%/50% or 10 percent.

Only half the females of the first generation have *H*, and only these will produce haploid sons; consequently the percent haploid males will be 10 percent from half the females, or 5 percent, and so on through subsequent generations, leaving a remainder which does not hatch. In all of the F_1 females, whether *Hh* or *hh*, 90 percent of the eggs are fertilized; 90 percent of these will be fertilized by diploid males, 10 percent by haploid males. The sexual offspring of the F_2 consist of 81 percent from diploid fathers of which half, 40.5 percent will be female and half will be male, and of 9 percent from haploid fathers and these will be all female (see preceding section).

Automatic frequency response occurs in this situation because the haploid males produce only daughters which, having in greater frequency the gene responsible for the production of haploid males, produce yet more of them. Table 1 illustrates how the frequency of gene *H*, and, after the initial drop, the frequency of haploid males, begin to increase.

A computer was used for calculations of subsequent generations and the results are illustrated in Figure 1. By the end of 50 generations, the frequency

TABLE 1

Changes in frequency of the gene H, *responsible for the development of unfertilized eggs to haploid males, during the first few generations of a new colony*

Generation	From fertilized eggs (90 percent)				From unfertilized eggs (10 percent)		Percent unhatched
	From diploid fathers		From haploid fathers				
	% Females	% Males	% Females (% of total females)		% Haploid males (Net % of all functional ♂♂)		
	Genotypes		Genotypes		Genotypes		
	Net female genotypes zygotic (Gametic)						
Parents	An *Hh* ♀ with an *hh* ♂ (i.e., one diploid male only).						
F_1	45%	45%			10%	(10%)	0%
	$.5Hh + .5hh$				$.5H + .5h$		
	$(.250H + .750h)$						
F_2	40.5%	40.5%	9% (18.2%)		5%	(5.8%)	5%
	$.0625HH + .375Hh + .5625hh$		$.125HH + .500Hh + .375hh$		$.5H + .5h$		
	$.0739HH + .3978Hh + .5283hh$						
	$(.273H + .727h)$						
F_3	42.4%	42.4%	5.2% (10.9%)		4.7%	(5.25%)	5.3%
	$.068HH + .387Hh + .545hh$		$.136HH + .500Hh + .364hh$		$.573H + .427h$		
	$.0754HH + .3993Hh + .5253hh$						
	$(.275H + .725h)$						
F_4	42.65%	42.65%	4.7% (9.9%)		4.7%	(5.22%)	5.3%
	$.0718HH + .3924Hh + .5358hh$		$.1576HH + .5328Hh + .3096hh$		$.579H + .421h$		
	$.0803HH + .4064Hh + .5134hh$						
	$(.283H + .717h)$						
F_5	42.65%	42.65%	4.7% (9.9%)		4.9%	(5.43%)	5.1%
	$.0758HH + .3994Hh + .5248hh$		$.1639HH + .5342Hh + .3018hh$		$.582H + .418h$		
	$.0845HH + .4127Hh + .5027Hh$						
	$(.291H + .709h)$						
F_6	42.6%	42.6%	4.8% (10.1%)		5.0%	(5.54%)	5.0%
	$.0803HH + .4064Hh + .5133hh$		$.1694HH + .5342Hh + .2964hh$		$.585H + .415h$		
	$.0891HH + .4194Hh + .4914hh$						
	$(.299H + .701h)$						
F_7	42.5%	42.5%	5.0% (10.5%)		5.1%	(5.66%)	4.9%
	$.0846HH + .4125Hh + .5019hh$		$.1749HH + .5341Hh + .2909hh$		$.588H + .412h$		
	$.0941HH + .4253Hh + .4797hh$						
	$(.307H + .693h)$						

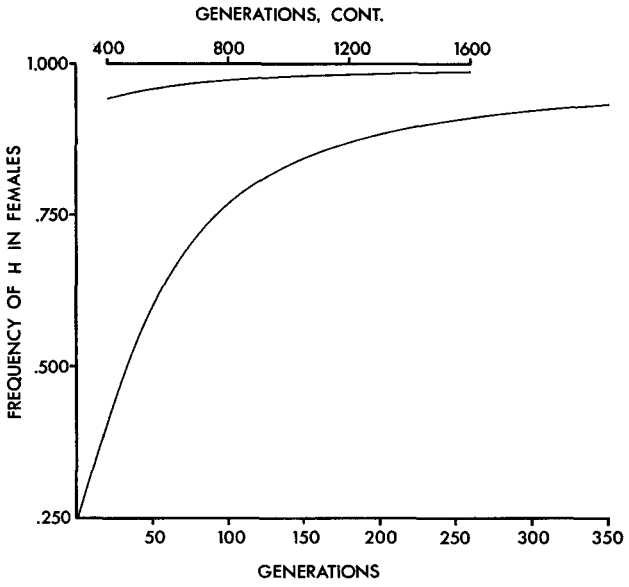


FIGURE 1.—The frequency of gene *H* during successive generations, from computer continuation of the calculations of Table 1.

of *H* has increased to 60 percent, and from about this point on, subcolonies would often be expected to be uniformly *H*. Within the original colony, uniformity is approached quite slowly above 80 percent and over 400 generations are required to give 95 percent *H*. Thus in colonies of any considerable size, a small percentage of *h* would persevere for a long time.

As will be shown below with other examples, the AFR is similar whether one founding member or one individual in an established colony is assumed heterozygous for the allele in question. The rate at which the frequency changes depends in part on the numbers involved; according to calculations made by MRS. RUTH B. GLASER, there is no lower threshhold here for an AFR although with sufficiently small percentages the response would be meaningless.

Complete uniformity for the gene *H* will not give a haplodiploid race. It will give a population in which all the unfertilized eggs develop and compete to poor advantage with the diploids. Undoubtedly selection would enter the picture to relieve the disadvantage, but this would be over a much longer period of time than that required for an AFR; since homozygosity must be assumed for the production of male haploidy, very little heritable variation would be expected to stem from heterozygosity already present, leaving such variation almost completely mutation-dependent; at even a moderately high mutation rate, long periods of time would be required. In the meanwhile, the AFR would assure the maintenance of the gene in the population.

The early stages of the evolution of male haploidy may therefore be looked upon as a time in which strains with varying percentages of haploid males are

present. The AFR's would be maintaining the "*H*" type genes, and selection would be gradually reducing the competitive differential. Two processes are of importance in the achievement of full male haploidy, i.e., a race or species with no more than sporadic diploid males; one of these involves the automatic replacement of diploid by haploid males, and will be considered in the next section; the other effects haploidy by means of successive AFR's.

If we assume that a gene of the *H* type has been established uniformly in a population and also for simplicity that selection has achieved equality of haploid and diploid males, a subsequent mutation increasing the percentage of unfertilized eggs will also show an AFR. As will be demonstrated in the next section, diploid males automatically disappear whenever the percentage of haploid males is one third (assuming haploid and diploid equality). The function of increasing the percentage of unfertilized eggs above one third is simply to change the sex ratio of haploid males to diploid females. Calculations showed that a gene increasing the percentage of haploids from $\frac{1}{3}$ to $\frac{1}{2}$ would show a positive AFR while from $\frac{1}{2}$ to $\frac{2}{3}$ the AFR would be negative.

Replacement of diploid by haploid males: In this section, it will be assumed that the haploid males are able to compete with the diploid on an equal basis. A series of relationships will be shown which vary according to the percentage of unfertilized eggs, assumed here to be exactly equal to the percentage of haploid males. A selective disadvantage could be entered into the model, if desired, simply by assuming a smaller percentage of the haploids.

Since the haploid males produce only daughters and since the females, it is assumed, produce a constant proportion of unfertilized eggs, there will be a tendency for the population to consist of nothing but diploid females and haploid males. This is accomplished rapidly simply as a function of these properties of the system, that is, without genetic change.

A model situation is worked out in Table 2. It is assumed that a colony is started by a diploid female and a diploid male. This female and all subsequent females uniformly produce one third unfertilized eggs which on maturity are haploid males on a par with the diploid males. The other two thirds of the eggs produced by the P_1 female are all fertilized by a diploid male and consequently half become daughters and half diploid sons. The ratio in the F_1 is thus 1:1:1 for diploid males, diploid females, and haploid males, or a 1:1 ratio for the two types of males.

Half of the gametes of the F_1 females will be fertilized by diploid males and half by haploid males. Of the two thirds of the eggs that are fertilized, the half that are fertilized by the haploid males will all be female zygotes; the half fertilized by diploid males will be half female and half diploid male. Thus of the sexual offspring $\frac{2}{3} \times \frac{1}{2}$ or $\frac{1}{3}$ will be daughters from haploid fathers; and $\frac{2}{3} \times \frac{1}{2} \times \frac{1}{2}$ or $\frac{1}{6}$ will be daughters from diploid fathers, and $\frac{1}{3} + \frac{1}{6}$, or $\frac{1}{2}$ will be female. The diploid males will be the other half of the half of the sexual offspring from the diploid fathers, or again $\frac{2}{3} \times \frac{1}{2} \times \frac{1}{2}$ or $\frac{1}{6}$. The ratio in the F_2 is thus 3:1:2 for diploid females, diploid males, and haploid males, or 1:2 for diploid to haploid males.

TABLE 2

Replacement of diploid by haploid males in a colony founded by a diploid pair; one third of eggs unfertilized and the haploid equal to the diploid males

	Diploid females	Diploid males	Haploid males	Ratio diploid: haploid males
P_1	one	one	. . .	. . .
(Fate of female gametes: 1/3 unfertilized; 2/3 fertilized, all by the diploid male)				
F_1 Proportion	1/3	1/3	1/3	1:1
Paternity	diploid	diploid	. . .	
(Fate of female gametes: 1/3 unfertilized; 2/3 fertilized, 1/2 by diploid, 1/2 by haploid males)				
F_2 Proportion	1/2	1/6	1/3	1:2
Paternity	1/6, diploid; 1/3, haploid	diploid	. . .	
(Fate of female gametes: 1/3 unfertilized; 2/3 fertilized, 1/3 by diploid, 2/3 by haploid males)				
F_3 Proportion	5/9	1/9	1/3	1:3
Paternity	1/9, diploid; 4/9, haploid	diploid	. . .	
F_n generation:				1:n

The relationship in subsequent generations is simple; the fraction of diploid males will be equal to $1/(n+1)$, and as n becomes large, the percentage of diploids approaches zero as a limit. Thus a colony which began with ⅓ of the total individuals or ½ the males as diploid soon has few or none remaining.

The following mathematical treatment has been worked out by GLASER. The ratio of diploid males to total males in any generation can be calculated according to the following method. Let N be this ratio; γ, the ratio of unfertilized eggs to total eggs, and a the *initial* ratio of diploid males to total offspring; in the example already cited, Table 2, $\gamma = ⅓$ and $a = ⅓$.

Now let $\gamma = ra/s$.

For the F_1, the ratio of diploid to total males will be simply $s/(r+s)$.

For the F_2, the ratio will be $N_2 = \dfrac{s}{(s\text{-}r) + r/a}$.

For the F_3, the ratio will be $N_3 = \dfrac{s^2}{s^2 + r(s\text{-}r) + r^2/a}$.

For the F_n, letting $i = n-1$, the ratio will be

$$N_{(i+1)} = \frac{s^i}{[s^i + r(s^{i-1} + r[s^{i-2} + \ldots + r(s^1\text{-}r)])] + (r^i/a)}.$$

For the example cited in Table 2, $s = r = 1$. The ratio of diploid males to total males will approach the value $1-r/s$ as a limit if $r < s$ but if $r \geq s$, then the limit will be zero. Thus the lowest proportion of haploid males which will be able to replace the diploids completely is one third, as in the example cited in Table 2. The rates of replacement of diploid by haploid males and the limiting values are shown in Figure 2 for colonies in which the females produce a constant percentage of unfertilized eggs, and the original mating was of a diploid female and diploid male; in short, like the example in Table 2 but also for other percentages of unfertilized eggs. For the limiting value of the AFR at 50 percent unfertilized eggs, the diploid males would be eliminated with very great rapidity, the per-

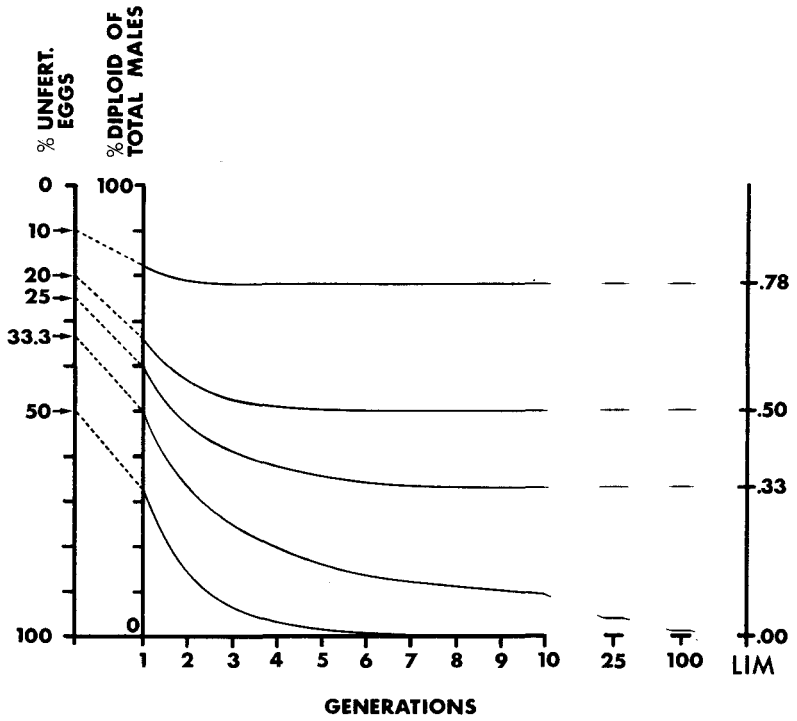


FIGURE 2.—Automatic replacement of diploid by haploid males. The first ordinate gives the percent of unfertilized eggs, assumed equal to the percent of haploid males. The second ordinate gives the percent of diploid males of the total males; the diploid males decrease in frequency, and the limit of each decrease is indicated on the last ordinate. The curve beginning with 33.3 percent unfertilized eggs is the same example as that in Table 2.

centage of diploid males being $1/(2^{n+1}-1)$. Although the replacement mechanism occurs with smaller percentages of haploid males, Figure 2 clearly indicates that it would be of relatively little significance with fewer than 10 percent haploid males, and undoubtedly of little importance during the early phases of the evolution of haplodiploidy.

The importance of the automatic replacement is that it provides a mechanism for achieving complete male haploidy. No genetic change is required to achieve this state; quite the contrary, the diploids are rapidly eliminated because the male haploids produce only daughters. In fact, the diploids will be eliminated at $\frac{1}{3}$ unfertilized eggs, a value lower than the limit of $\frac{1}{2}$ which will be reached by automatic frequency responses.

This is not to gainsay that genetic mechanisms of various sorts do not play a part in the fixation of male haploidy. For example, translocations of the X and autosomes would prevent recurrence of diploid males through simple nondisjunction.

Some further aspects of male haploidy: It is of interest to note several other

relationships. In the model used above, the female parent alone was heterozygous. In male haploidy, the frequency of a gene of no selective differential will show in a similar model a gradually diminishing oscillation. If the original parents are *Aa* and *A*, the frequency of *a* in the female is ½; in the offspring, two classes of females will be present, *Aa* and *AA*, and the frequency in the F_1 is ¼. In the F_2, it is ⅜. The frequency in the female is equal to $\frac{1}{3} + 1/[6 \times (-2)^n]$; as n increases, the second term approaches 0 and the frequency of the gene approaches ⅓. This oscillation may occur as a component in various series involving male haploidy, and obscure other relationships.

The Hardy-Weinberg formulation, $p^2\ AA + 2pq\ Aa + q^2\ aa$, seems applicable at equilibrium, even though it gives only the frequencies of female genotypes. The frequencies of male genotypes are, however, identical to that of the female gametes of the preceding generation. Since male genotypes are equivalent to male gametes, these must equal the female gametes since by definition the population is in equilibrium.

It has sometimes been suggested that male haploid systems have an advantage because the haploids are killed by recessive lethals, thus eliminating the lethals from the population. The same model can be assumed for a colony started by the diploid parents, $Ll \times LL$, or the haplodiploids, $Ll \times L$, in which *l* is a recessive lethal. It will be assumed that the sex ratio is 1:1 in both instances. The frequency of the lethal in the diplodiploid colony is $1/(n + 3)$ and in the haplodiploid is $1/2^{n+1}$, n being the generation number following the P_1 given above. The haplodiploids obviously lose the lethal at a much faster rate. None of the female zygotes in the haplodiploid are *ll*, so no productivity is lost because of the lethal. In the diplodiploid, 1/16 of all the zygotes, thus 1/16 of the females are *ll* in the F_2. This is a decrease in productivity of about 6 percent. In the next generation, 1/25 of the zygotes are *ll*, a decrease of 4 percent. The percentage rapidly grows smaller and at the eighth generation only 1 percent are *ll*. Thus the haplodiploids would have a slight advantage over the diplodiploids for only a few generations. (This advantage would be further reduced if the greater loss of males on the part of the haplodiploids resulted in some of the females remaining unfertilized.) For a fair comparison, both must be assumed to be inbred, with little heterozygosity, and such advantage as accrued to the haplodiploids would recur only on the advent of lethal or deleterious mutations.

The ability to eliminate lethals rapidly with no loss of productive females may have enabled haplodiploid groups to evolve rapidly. They could exploit a high mutation rate without loss of productivity from the disadvantageous changes which would inevitably accompany the desirable mutations. On the other hand, the screening of mutants from an inbred diplodiploid would seemingly be of little value in favoring the establishment of newly appearing male haploids; to the contrary, the loss of the haploid males because of the lethals would seriously impair the AFR mechanism for a few generations. A balanced lethal system would be one of the ways in which male haploidy could be prevented from becoming established.

Lecano-Diaspidid Series

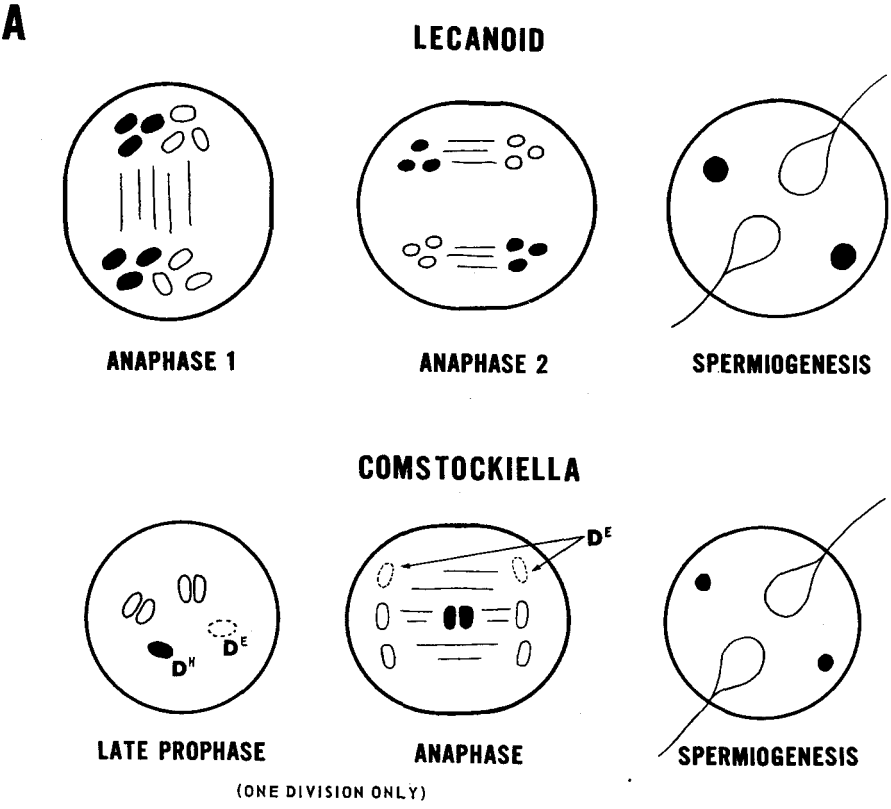
The lecano-diaspidid evolutionary series of the coccids consists of four chromosome systems (BROWN and MCKENZIE 1962; BROWN 1963). The differences are confined to the male sex, the female remaining quite orthodox in regard to chromosome behavior. The series begins with a typical XX–X0 system but the next step is to the highly unusual mealy-bug, or lecanoid, system (HUGHES-SCHRADER 1948). The Comstockiella system is the third in the series (BROWN 1963) and the series ends with the diaspidid (BROWN and BENNETT 1957; BENNETT and BROWN 1958). Since the systems have all been described in detail elsewhere, only limited information, sufficient to make them intelligible for present purposes, will be provided here.

In the lecanoid system the paternal chromosome set becomes heterochromatic in early embryogeny and remains so during development. Spermatogenesis consists of two divisions (Figure 3A); during the first, the heterochromatic and euchromatic (i.e. paternal and maternal) sets both divide equationally; during the second, the two types are segregated into opposite daughter nuclei. Of the resultant four products, only the two nuclei derived from the maternal or euchromatic set form sperm while the other two, derived from the paternal or heterochromatic set, degenerate during spermiogenesis. The lecanoid male thus breeds like a haplodiploid male, transmitting only the maternal chromosomes (SCHRADER 1923; HUGHES-SCHRADER 1935, 1948; BROWN and NELSON-REES 1961; NELSON-REES 1962).

Early development in the Comstockiella system is like that in the lecanoid. Spermatogenesis consists of but one division (Figure 3A). There are two types of chromosome behavior, that of the D pair, and that of the remainder, to be described first. Prior to or during early prophase, the distinction between the eu- and heterochromatic states is lost, and the respective homologues pair with each other, one member of each pair originating from the eu-, the other from the heterochromatic set. At anaphase, the pairing partners separate to opposite poles, apparently at random, and both resulting daughter nuclei form sperm. The D chromosomes, always but one pair regardless of total chromosome number, maintain an essentially lecanoid type of behavior. The euchromatic D derivative (D^E) divides equationally at anaphase and each daughter half is included in one of the sperms; the heterochromatic D derivative (D^H) also divides but the halves are excluded or ejected from the daughter nuclei. Thus, as with all the chromosomes in the lecanoid system, the D pair yields four derivatives, two of which are incorporated in sperm while the other two degenerate (BROWN 1957, 1963).

By contrast, the diaspidid system is quite simple; the paternal chromosomes are eliminated at late cleavage and the male proceeds thereafter to develop as a haploid. Spermatogenesis consists of a single, equational division (BROWN and BENNETT 1957; BENNETT and BROWN 1958).

In regard to hereditary transmission, the XX–X0 and Comstockiella systems behave as diploids, transmitting both maternal and paternal sets of chromosomes, while transmission from the lecanoid and diaspidid males is that of a haploid since only the maternal set is passed on. As mentioned in the introduction, the



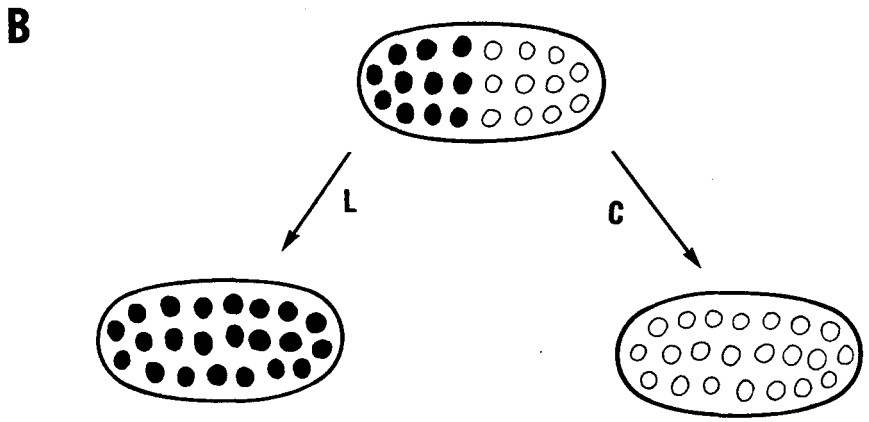


FIGURE 3.—The lecanoid-Comstockiella relationship. **A.** Spermatogenesis in the two systems. In the lecanoid system both the euchromatic (light) and heterochromatic (dark) chromosomes divide equationally in the first division; in the second, the two types are segregated; only the two euchromatic derivatives form sperms. In the Comstockiella system, homologous chromosomes pair at prophase and separate at anaphase of the single division; both products form sperms; the D^E chromosome divides equationally while its heterochromatic homologue, D^H, is eliminated. **B.** Diagrams of testes. In the original condition, the lecanoid system occurs in 50 percent of the cysts (dark) and the Comstockiella system in the other 50 percent (light). An *L*-type gene converts all the cysts to the lecanoid system; a *C*-type converts them all to the Comstockiella. (See text and Table 3 for expected transmissions.)

series thus alternates, diploid-haploid-diploid-haploid, in regard to transmission.

The evolutionary series of four steps includes three transitions. Two of these are diploid-to-haploid changes and should thus be expected to behave in regard to automatic frequency response like male haploidy as described in the previous section (BROWN 1963). In the first of these changes, XX–X0 to lecanoid, the gene showing an AFR would be active in the mother and responsible for altering certain but not all eggs so that the paternal chromosome set would become heterochromatic. Even though fertilization occurs, the net result is haploid transmission; whether the paternal set is XA or 0A, it is heterochromatized and finally eliminated, resulting in the transmission of the maternal XA only. During the early stages of evolution of the lecanoid system, both X0 males and the heterochromatic type would be expected and the latter would replace the former because all of their non-heterochromatized offspring would be female. Recent evidence of NUR (1963) shows that heterochromatization in the embryos in at least one instance is quite definitely under the control of maternal developmental processes. It does not matter, however, in regard to the AFR, how the maternal gene might produce its effect in the developing embryo.

The second diploid-haploid change is the transition from the Comstockiella to the diaspidid system. Because sex chromosomes had been eliminated in this evolutionary series with the invention of the lecanoid system, these did not complicate this step which was exceedingly simple: slightly before the embryonic stage at which the chromosomes are heterochromatized in the Comstockiella system, they are completely eliminated in the diaspidid. The gene showing a positive AFR under this circumstance would effect a complete elimination rather than a heterochromatization of the paternal chromosomes. As far as cytology is concerned, the diaspidid system could have been derived presumably as easily from the lecanoid as from the Comstockiella and a change of this type, from lecanoid to a more complete male haploidy, had indeed been expected by several workers in the field (SCHRADER and HUGHES-SCHRADER 1931; BROWN 1958). However, an AFR will not appear in a haploid-to-haploid change. In the transition from Comstockiella to diaspidid, however, the change was diploid to haploid and an AFR would be expected.

The lecanoid-Comstockiella relationship: The lecanoid is the more primitive system, spanning the range from the unspecialized mealy bugs to the advanced asterolecaniids; the Comstockiella system occurs only in the highly specialized armored scales and in certain more primitive forms believed to be in the immediate ancestry of the armored scales (BROWN and MCKENZIE 1962; BROWN 1963; NUR and BROWN unpublished).

The two systems are exactly alike until spermatogenesis. In several species, both systems occur, and often in the same testis (BROWN 1963). They are cyst specific, and usually large sections of a testis are uniform, but no relationship has yet been observed between the position in the testis and chromosome system. A mixed testis of this sort is an excellent point of departure for demonstrating the automatic frequency responses shown between the two systems (Figure 3B).

It should be recalled at this juncture that only the maternal chromosomes are

transmitted in the lecanoid system while those of both parents are handed down in the Comstockiella (except for the D pair). If half the cysts of a testis are lecanoid and half Comstockiella, then the maternal chromosomes will be transmitted from 100 percent × ½ and 50 percent × ½, or in ¾ of the sperm, while the paternal will be transmitted from only the Comstockiella half, 50 percent × ½, or in ¼ of the sperm. Transmission from a mixed testis for any heterozygote would also follow this rule.

Four types of genes may be compared in regard to AFR. For the sake of simplicity all will be considered to make complete changes. An *L*-type allele is responsible for converting the mixed testis to one which has only the lecanoid system; likewise a *C*-type allele converts the mixed testis to complete Comstockiella. Each of these two types may be active in the euchromatic or heterochromatic set. Although genetically inert as tested by dominant lethality (BROWN and NELSON-REES 1961) and by mutant genes (BROWN and associates, unpublished) the heterochromatic set is certainly not devoid of influence on development or fertility (NELSON-REES 1962; NUR and CHANDRA 1963).

The case of *L* active in the euchromatic set, L^E, will now be considered. Regardless of the types of cyst, the two homozygotes L^EL^E and ll can transmit only L^E and l respectively. The two heterozygotes differ according to whether L^E is of maternal, l of paternal origin, symbolized as $L^E(l)$, or vice versa, $l(L^E)$. Since *L* is active in the euchromatic, or maternal set, it will be expressed in the $L^E(l)$ heterozygote, and the cysts here will all be lecanoid. In the $l(L^E)$ heterozygote, the *L* allele is in the paternal, or heterochromatic set, and inactive; the cysts will remain in the original ratio, half lecanoid and half Comstockiella. Since all the cysts of the $L^E(l)$ heterozygote are lecanoid and therefore transmit only the maternal chromosomes, the $L^E(l)$ individual will transmit only L^E. In the $l(L^E)$ individual, half the cysts are lecanoid, these will transmit only l; the other half are Comstockiella, and these will transmit half l and half L^E; the gametic proportions for the $l(L^E)$ heterozygote are thus 75 percent l to 25 percent L^E. If the two classes of heterozygotes are equal in frequency in a population, then the gametic ratio for the heterozygotes combined is 125 percent L^E to 75 percent l. Such differential yield from the two types of heterozygote results in a positive AFR in which L^E rapidly increases.

If on the other hand, an *L*-type gene is assumed active in the heterochromatic set, symbolized as L^H, then its frequency will rapidly decrease. The $L^H(l)$ heterozygote shows no change from the original mixed testis, and thus yields 75 percent L^H and 25 percent l. In the $l(L^H)$ heterozygote, the gene L^H is now active, converting all the cysts to the lecanoid system and thus blocking completely its own transmission since it is of paternal origin. The $l(L^H)$ heterozygote thus transmits 100 percent l. The heterozygotes, if equal, will give a combined yield of 125 percent l to 75 percent L^H; and the allele L^H will be rapidly eliminated from the population. The AFR is considered to be negative in this case rather than that of l to be positive because it is of interest to compare the AFR's of genes responsible for the various changes in development rather than of those of no effect.

Similar AFR's are shown by the *C* factors except that the situation is reversed,

C^H shows the positive and the C^E the negative AFR. If C is active in the heterochromatic set, then the $C^H(c)$ heterozygote will show no change from the original, and transmit only C^H from the lecanoid cysts and 50 percent of each from the Comstockiella cysts or 75 percent C^H and 25 percent c. The $c(C^H)$ heterozygote will have only Comstockiella cysts and the yield for the entire testis will be 50 percent c and 50 percent C^H. When equal, the two classes of heterozygotes give a combined yield of 125 percent C^H and 75 percent c. The differential transmission results in a positive AFR.

Conversely, the C factor active in the euchromatic set, C^E, yields a negative AFR. These relationships are summarized in Table 3; all of the combined heterozygotes yield the same, 125 percent of one sort, 75 percent of the other, and thus the rate of increase or decrease in all of the AFR's will be about the same. The rate of change in gene frequency following these changes is quite rapid (Figure 4). The exact percentages will show minor differences in the various models for various reasons; for example, in the model beginning with a heterozygous female, she must transmit to a grandson, rather than to a son, for the first expression of a gene active in the heterochromatin. The strength of the AFR here depends on the extent of the change; a gene responsible for a change from 50 percent to 75

TABLE 3

Combined yield from heterozygotes for genes shifting the ratio of cysts from half lecanoid and half Comstockiella to all one or the other‡

L-type genes, to 100 percent lecanoid cysts				
1. L^E, active in euchromatin; positive AFR				
genotypes	L^E (L^E)*	L^E (l)	l (L^E)	l (l)
cysts	all lec	all lec	½ & ½	½ & ½
gametes	100% L^E	100% L^E	25% L^E; 75% l	100% l
combined yield of heterozygotes:† 75% l, 125% L^E				
2. L^H, active in euchromatin; negative AFR				
genotypes	L^H (L^H)	L^H (l)	l (L^H)	l (l)
cysts	all lec	½ & ½	all lec	½ & ½
gametes	100% L^H	25% l; 75% L^H	100% l	100% l
combined yield of heterozygotes:† 75% L^H, 125% l				
C-type genes, to 100 percent Comstockiella cysts				
3. C^E, active in euchromatin; negative AFR				
genotypes	C^E (C^E)	C^E (c)	c (C^E)	c (c)
cysts	all Com	all Com	½ & ½	½ & ½
gametes	100% C^E	50%c; 50%C^E	25%C^E; 50%c	100% c
combined yield of heterozygotes:† 75% C^E, 125% c				
4. C^H, active in heterochromatin; positive AFR				
genotypes	C^H (C^H)	C^H (c)	c (C^H)	c (c)
cysts	all Com	½ & ½	all Com	½ & ½
gametes	100% C^H	25%c; 75%C^H	50%c; 50%C^H	100% c
combined yield of heterozygotes:† 75% c, 125% C^H				

* The genes in the paternal, heterochromatic set are cited in parentheses.

† Combined yields quoted on the assumption that the two sorts of heterozygotes are equal which they would not be if an AFR were in progress. This calculation, however, provides a quick estimate of the sign and strength of the AFR.

‡ The chromosome systems and the changes in the cyst ratios used in the model are diagrammed in Figure 3.

percent, for example, rather than to the 100 percent used in the model would show a slower response.

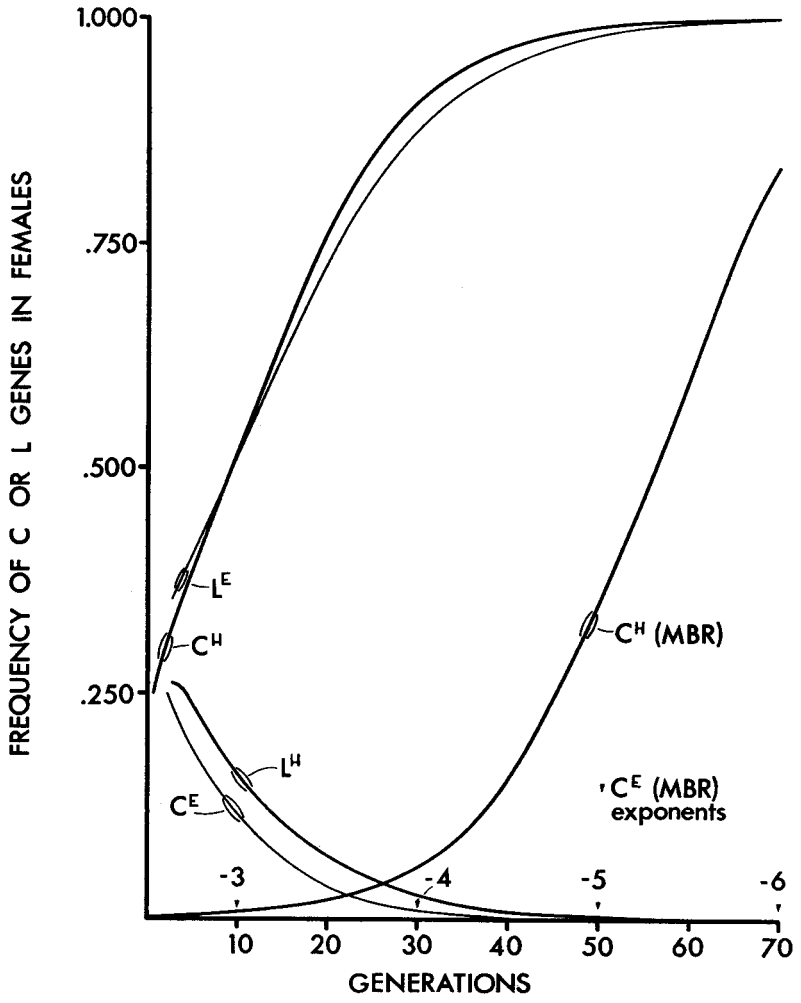


FIGURE 4.—Curves of automatic frequency responses following changes from a half-and-half testis to one either all Comstockiella (C) or all lecanoid (L). Gene frequencies are shown only for the female sex. When the gene is active in the euchromatin (^{E}), the AFR is negative for C^E and positive for L^E; when active in the heterochromatin (^{H}), the AFR is positive for C^H and negative for L^H. The four curves beginning near the .250 frequency are from the model in which the colony is founded by two parents and the female is heterozygous for the gene in question; the marked fluctuations for the first two or three generations have been omitted. The curve and points marked MBR are from the model in which the gene in question first occurs in heterozygous state in one female member out of 100 such in the colony; only the Comstockiella example is given. If positive, the MBR curve rises rapidly after a gradual start. The curve for the negative AFR is not shown, the triangular points indicate the generation at which the frequency dropped to 1×10^{-3}, etc., the values thus rapidly become meaningless for colonies of limited size.

Calculations were also made on the assumption that the gene in question appeared in heterozygous state in one female out of 100 in the colony, thus one mutant gene out of 200 or a P_1 frequency in the females of 0.005. Contributions to successive generations are presumed to be about equal from the various parents. For the negative AFR to have actual meaning, the colony would have to increase in size; the calculated frequencies of C^E in the female drop to 0.001 in 10, and 0.0001 in 30, generations. Thus the probability is quite high that under such circumstances a C^E gene would be completely lost from a small colony in a few generations (Figure 4).

If, however, the gene shows a positive AFR, its increase in frequency is quite rapid. Within about 45 generations, the frequency of C^H in the females has risen from 0.005 to about 0.25 or approximately where it would be after one generation in the previous model (Figure 4). An AFR can be proportionately as effective when the mutation occurs in a colony member as when it is assumed present in one of the founders.

The cytological aspects of the evolution of the Comstockiella from the lecanoid system have been discussed in detail elsewhere (BROWN 1963) and will not be repeated here. Suffice it to say that the Comstockiella system probably first appeared as an altered chromosome mechanism in a few cysts in an otherwise lecanoid testis. Calculations were made of the change in frequency of a *C*-type gene, active in the heterochromatin and responsible for changing 5 percent of the cysts from the lecanoid to the Comstockiella system. Under this circumstance about 1030 generations are required to reach a frequency of 95 percent when the gene first appears in one heterozygote among 100 females (Figure 5). This example, on comparison with the "C^H(MBR)" curve of Figure 4 also illustrates the proportionality between rate of change in gene frequency and magnitude of developmental change.

When both the Comstockiella and lecanoid systems occur to appreciable frequency, it must be concluded that the genetic control for each has been highly

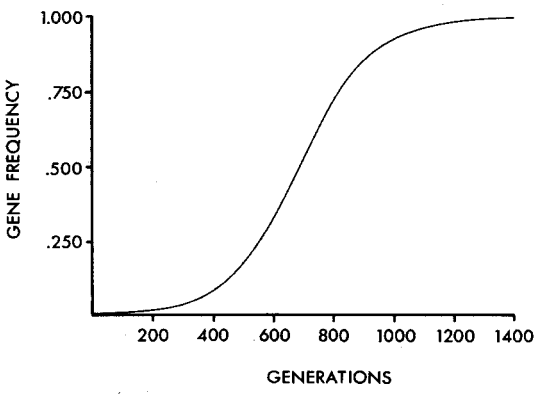


FIGURE 5.—Automatic frequency response curve, for the females, of a gene converting 5 percent of cysts from lecanoid to Comstockiella, active in the heterochromatic set, and first appearing in heterozygous state in one female out of 100 females in a colony.

refined and genes which change the proportion of the two types may be nothing more than "switch" genes, those canalizing development along one track or another. Since both systems have been perfected, no sterility would be expected regardless of the proportion of the two types. Cytological examination has revealed normal spermiogenesis occurring just as regularly in the species with both systems as in those with only one. On the contrary, when the Comstockiella system first appeared, the genes in question could not have been switch genes but must have been directly concerned with altering chromosome behavior. Furthermore, the alterations were not necessarily successful and some sterility could easily have resulted; the sterility would have operated to slow down a positive AFR, as well as place the individuals with the new mutant genes at some selective disadvantage.

DISCUSSION

Direction of evolutionary change: Positive automatic frequency responses would be expected in transitions from one to the next of the following coccid chromosome systems:

(1) XX–X0 → lecanoid ⇄ Comstockiella → diaspidid
(2) XX–X0 → haplodiploid
(3) XX–X0 → diaspidid

The first two of these sequences are known, the third is not. The cytological changes required to produce a diaspidid system from a Comstockiella are probably simpler than those required for true male haploidy and the reverse may well be true for the derivation of the diaspidid from the XX–X0.

The only direct evidence of the significance of the AFR is provided by the first sequence. This order is exactly the same as that which the taxonomic evidence indicates (BALACHOWSKY 1942). This evidence has been examined in considerable detail recently (BROWN 1959; BROWN and MCKENZIE 1962; BROWN 1963) and will not be repeated here.

The AFR's for the change from the lecanoid system to the Comstockiella and the reverse are of exactly the same strength (Table 3), and the two systems are, or can be identical until spermatogenesis. A complex situation, rather than a clean transition might well be expected at this juncture. Of the various species surveyed whose morphology indicates that they must have had ancestors with Comstockiella chromosome behavior, one is pure lecanoid, eleven are pure Comstockiella, and six are mixed (BROWN unpublished). It should also be noted that the supposedly pure species might not prove to be so on more extensive examination. On the other hand, it seems likely that the apparently several (or at least two) derivations of the diaspidid from the Comstockiella system (BROWN and MCKENZIE 1962) must have depended upon the prior establishment of a pure Comstockiella system; otherwise the achievement of a male-haploid-like system would have been very much simpler by the already partially accomplished reversion to lecanoid.

It has been suggested that pure Comstockiella species may have resulted from

the genes responsible for the forward changes being in advance of those responsible for reversion to lecanoid behavior (BROWN 1963). With low mutation rates, and a colonial population pattern, races would tend to be established each with its own characteristic proportion of the two systems. Any factor tending to promote the one at the expense of the other would shift the proportions of the two types. If the mutations shifting the percentage toward more Comstockiella cysts occurred more readily because they were active in the heterochromatic state, then more species with the Comstockiella system would be expected. If evolution for any of several reasons, such as greater mutability in the heterochromatic state, were faster with a Comstockiella system, again, more species with this system would be expected. Relatively little is yet known about the Comstockiella system and there may be in this system properties which would permit stabilization by preventing successful reversion to the lecanoid system.

Suffice it to say, therefore, that even if both the AFR's are positive and equal, the two systems may have quite dissimilar potentialities otherwise for evolutionary progress. The one breeds apparently quite simply as though stemming from an unfertilized egg; the other breeds as a diploid and achieves that diploidy by a reversal of heterochromatization. Thus two entirely different systems are counterposed opposite the equal AFR's and neither a 50 percent equilibrium, nor any equilibrium at all could be predicted with any certainty at the present time.

Selection and chance fixation: The long range influences of selection may be distinguished from the immediate. Long range responses to selection have undoubtedly been of considerable importance in the success or failure of species; one example of a possible difference in this regard between male haploid and conventional systems is that described above of the very effective screening of recessive lethals by the haploid males, and further consideration of long term effects will not be undertaken here.

It has long been difficult to understand how new systems, in the crude state of their first emergence, could compete successfully with those already in existence. In some instances, as with the evolution of the Comstockiella system, gradual improvement may have occurred from the time of the first few changes. In others, as with male haploidy itself, there can be no recourse to gradualism.

In male haploidy, once individuals capable of at least some matings have appeared, AFR and selection will interact in two ways. First, as shown by the model cited in the first part of the paper, AFR will shield the responsible gene against adverse selection enabling it to increase in frequency in spite of stringent selective differentials. Secondly, the increase in frequency will increase markedly the probability that subsequent mutations, potentially capable of enhancing the male haploid, will occur where effective. If only the original small percentage of male haploids continued to be produced, i.e., the gene responsible did not spread through the population, the chances of a second mutation becoming associated with the first would be extremely small indeed. And it should be recalled that inbreeding and its consequent homozygosity preclude a store of genetic variability; progress must depend upon mutation.

During the early stages in the evolution of male haploidy, two processes will

be at work. AFR's will tend to spread through the population the genes primarily responsible for the production of haploid males from unfertilized eggs whether these genes are responsible for the first appearance or for a later increase in percentage (to the specified limit). While these genes are increasing, selection will operate to increase the frequency of other mutant genes responsible for bringing the haploid closer to par with the diploid males. At some stage, automatic replacement of the diploid by haploid males would help to convert the race or species to one which is purely haplodiploid.

Undoubtedly the random effects on gene frequencies due to fluctuations in population size are of special importance with a colonial population structure since any one colony persists for but a short time and the new colonies are probably founded most often by one pair. Under such circumstances homozygosity for the most prevalent allele in the old colony will frequently occur in the new. The typical AFR curves approach 0 and 100 percent asymptotically; owing to random fixation however, these end points must be reached in subcolonies sooner than the curves indicate, and the small remaining percentage of the other allele undoubtedly disappears with disappearance of the original colony. The interaction here may be defined as resulting in a relatively abrupt achievement of the asymptotic limits for individual colonies; if there are hangers-on over a long period of time, they are probably chance continuations of mixed colonies or of different, independent races, rather than small percentages.

Meiotic drive and AFR: According to SANDLER and NOVITSKI (1957), when heterozygotes fail to produce the two kinds of gametes with equal frequency, this "pattern of behavior will drastically alter frequencies of alleles in a population; where such a force, potentially capable of altering gene frequencies, is a consequence of the mechanics of the meiotic divisions, we suggest that the name *meiotic drive* be applied." "Genic meiotic drive," governed by factors on the chromosome, was distinguished from "chromosomal meiotic drive" attributable to structural differences. Furthermore, meiotic drive provided a mechanism by which deleterious genes might be increased in frequency. More recently HIRAIZUMI, SANDLER, and CROW (1960) have stated that all known cases of meiotic drive are associated with a reduction in fitness, perhaps because those in which the drive has resulted in fixation are no longer recognizable as such.

Of the several possible ways of escape from an unfavorable meiotic drive which have been suggested by various authors, that described by SANDLER and NOVITSKI (1957) is especially pertinent here, "If the adaptive value of a species is being lowered by genes subject to meiotic drive, then there may be some immediate positive selective value to factors that drastically alter the nature of meiosis, with the result that the specific events on which meiotic drive depends are eliminated or changed in some way."

Adaptation to homozygosity must precede the evolution of male haploidy and similar systems. Meiotic drive would presumably be less apt to occur in species so adapted since inbreeding *per se* would tend to eliminate the heterozygosity on which the drive depends. Although certain developmental changes, including those of meiosis, would simultaneously result in both an AFR and a release from

meiotic drive, it seems likely that the two processes would appear on quite distinct backgrounds, with AFR's associated with inbreeding and meiotic drives with heterozygosity.

It is probably proper to view the two processes as parallel, or rarely convergent, the latter when the same asymmetry in behavior could have been achieved either by factors on the chromosome in question or by developmental changes. An example of convergence may perhaps be found in the initial phases of the evolution of the Comstockiella system from the lecanoid (BROWN 1963) if indeed it is fair to apply the concepts of meiotic drive to the unilateral lecanoid system.

If a meiotic drive resulted in successful fixation, evidence of the process disappeared along with the heterozygotes. (Examples of fixation which conform to expectation from meiotic drive may, however, be obtainable from chromosome structural changes occurring during evolution.) On the other hand, developmental alterations which become permanently established continue to provide evidence of the AFR's which must have occurred during their evolution.

The author is indeed indebted to MRS. RUTH B. GLASER for her helpful collaboration in programming the AFR models for the computer and for the derivation of the general formulas for the automatic replacement of diploid by haploid males.

SUMMARY

Automatic frequency responses (AFR's) occur when genes responsible for altering the life cycle, including meiosis, are thereby either increased or decreased in frequency. There are no known examples of specific genes exhibiting an AFR; the treatment is, therefore, completely theoretical.

An AFR would be expected to occur in the evolution of male haploidy and in each of the transitions of a 4-stage evolutionary sequence in the coccids. The chromosome systems in this sequence alternate in regard to hereditary transmission: diploid-haploid-diploid-haploid. This unusual sequence does, however, conform to expected positive AFR's, providing the only evidence yet available from nature of the significance of the process.

Changes in gene frequency depend in part upon the extent of developmental change induced and the greater the change, the more rapid the AFR. Even with the relatively small changes expected at an evolutionary beginning, the changes in frequency occur with great rapidity viewed against an evolutionary time scale.

The interaction between AFR's and selection is complex; AFR's may increase the frequency of a gene against considerable selection pressure. Random fixation, of special importance in such situations as the colonial population patterns of the coccids, would tend to bring the AFR's more abruptly to their asymptotic limits.

Automatic frequency response parallels meiotic drive; in most instances the two processes are distinctly different in mode, but alike in their influence on gene frequency. In a few cases, where the two processes seem to converge, it is difficult to determine whether the gene in question governs the behavior of the chromosome directly (meiotic drive) or indirectly via its prior influence on the milieu (AFR).

LITERATURE CITED

BALACHOWSKY, A. S., 1942 Essai sur la classification des cochenilles (Homoptera: Coccoidea). Grignon Ec. Natl. Agr. Ann. (sér. 3) **3**: 34–48.

BENNETT, F. D., and S. W. BROWN, 1958 Life history and sex determination in the diaspine scale, *Pseudaulacaspis pentagona* (Targ.) (Coccoidea). Can. Entomologist **90**: 317–325.

BROWN, S. W., 1957 Chromosome behavior in *Comstockiella sabalis* (Comstk.) (Coccoidea-Diaspididae). Genetics **42**: 362–363. —— 1958 Haplodiploidy in the Diaspididae—confirmation of an evolutionary hypothesis. Evolution **12**: 115–116. —— 1959 Lecanoid chromosome behavior in three more families of the Coccoidea (Homoptera). Chromosoma **10**: 278–300. —— 1963 The Comstockiella system of chromosome behavior in the armored scale insects (Coccoidea: Diaspididae). Chromosoma **14**: 360–406.

BROWN, S. W., and F. D. BENNETT, 1957 On sex determination in the diaspine scale *Pseudaulacaspis pentagona* (Targ.) (Coccoidea). Genetics **42**: 510–523.

BROWN, S. W., and H. L. MCKENZIE, 1962 Evolutionary patterns in the armored scale insects and their allies (Homoptera: Coccoidea: Diaspididae, Phoenicococcidae, and Asterolecaniidae). Hilgardia **33**: 141–170A.

BROWN, S. W., and W. A. NELSON-REES, 1961 Radiation analysis of a lecanoid genetic system. Genetics **46**: 983–1007.

HIRAIZUMI, Y., L. SANDLER, and J. F. CROW, 1960 Meiotic drive in natural populations of *Drosophila melanogaster*. III. Populational implications of the segregation-distorter locus. Evolution **14**: 433–444.

HUGHES-SCHRADER, S., 1935 The chromosome cycle of Phenacoccus (Coccidae). Biol. Bull. **69**: 462–468 —— 1948 Cytology of coccids (Coccoidea-Homoptera). Advan. Genet. **2**: 127–203.

NELSON-REES, W. A., 1962 The effects of radiation damaged heterochromatic chromosomes on male fertility in the mealy bug, *Planococcus citri* (Risso). Genetics **47**: 661–683.

NUR, U., 1963 Meiotic parthenogenesis and heterochromatization in a soft scale, *Pulvinaria hydrangeae* (Coccoidea: Homoptera). Chromosoma **14**: 123–139.

NUR, U., and H. S. CHANDRA, 1963 Interspecific hybridization and gynogenesis in mealy bugs. Am. Naturalist **97**: 197–202.

SANDLER, L., and E. NOVITSKI, 1957 Meiotic drive as an evolutionary force. Am. Naturalist **91**: 105–110.

SCHRADER, F., 1923 A study of the chromosomes in three species of Pseudococcus. Arch. Zellforsch. **17**: 45–62.

SCHRADER, F., and S. HUGHES-SCHRADER, 1931 Haploidy in metazoa. Quart. Rev. Biol. **6**: 411–438.

WHITE, M. J. D., 1954 *Animal Cytology and Evolution*, 2nd edition. Cambridge University Press.