INTERSPECIFIC HYBRIDS AND SPECIATION IN THE GENUS ACHETA (ORTHOPTERA, GRYLLIDAE)[1]

R. S. BIGELOW[2]

Abstract

Acheta rubens produced interspecific hybrids with both the "northern spring species" (NSS) and *assimilis* in the laboratory, but not with *pennsylvanicus; assimilis* crossed with both *rubens* and *pennsylvanicus* but not with NSS; NSS crossed only with *rubens; pennsylvanicus* crossed only with *assimilis*. All other attempts to cross the four species failed. Over 2500 *assimilis* × *rubens* hybrids were produced within $2\frac{1}{2}$ months; less than 1000 *rubens* × NSS hybrids were produced over about 18 months. The developmental rate of males carrying *rubens X* chromosomes was extremely slow in both *rubens* × *assimilis* and *rubens* × NSS hybrids; hybrid males carrying either *assimilis* or NSS *X* chromosomes, and all hybrid females, developed at normal rates, or at rates intermediate between those of the parental species. The songs of certain hybrid males were intermediate between those of the parental species in certain features but resembled the song of one parental species in other features. Fertile backcross males and females were produced by *rubens* × NSS hybrid females but the viability and fertility of the offspring of these specimens were abnormally low; both backcross and F_2 nymphs have been produced by *rubens* × *assimilis* hybrids. The incubation periods of hybrid eggs laid by *pennsylvanicus* females were less than half those of normal *pennsylvanicus* eggs at the same temperature.

Introduction

The solution of taxonomic problems in North American crickets of the genus *Acheta* has proved to be difficult, if not impossible, when approached from the classical (i.e. gross morphological) point of view. Rehn and Hebard (5) relegated 47 names to synonymy under *Acheta assimilis* F., and thus proclaimed the failure of a considerable body of previous work on the genus, nearly all of which had been based on purely morphological macroscopic evidence. Fulton (4), using biological evidence, revealed the presence in the southeastern United States of four reproductively isolated entities. Alexander (1) raised these entities to full specific status and described a new species, chiefly on the basis of similar biological evidence. A sixth species will be named by Alexander and Bigelow (2), but as the name will not be available before the present paper goes to press, this species will be referred to here as "NSS" (northern spring species).

The six *Acheta* species (excepting *A. domesticus* (L.)) now known to inhabit North America east of the Mississippi and north of Florida are:

A. pennsylvanicus (Burmeister). Northern fall field cricket.
A. firmus (Scudder). Beach cricket.
A. rubens (Scudder). Triller field cricket.
A. vernalis (Blatchley). Northern wood cricket.
A. fultoni Alexander. Southern wood cricket.
NSS. Northern spring field cricket.

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[2]Department of Entomology and Plant Pathology.

It is now clear that the genus *Acheta* in North America comprises a complex of very closely related species which have not diverged far beyond the point where hybrids can no longer form an effective bridge for gene interflow. A fuller understanding of these species should therefore throw light on features of reproductive isolation that are more or less peculiar to this group, as well as on the speciation process in general. Studies by Alexander (unpublished) have revealed the presence of at least 10 additional species in North America and future studies of the genus in the West and Southwest, and in the West Indian archipelago, will probably show that these regions also contain *Acheta* species that are unknown at the present time. The complex as a whole should prove to be a rich source of information on insect speciation.

This paper is an account of crossing tests between three of the species listed above and *Acheta assimilis* F. from Jamaica. The results of these tests, in the form of interspecific hybrids, bear directly on the extent and kinds of reproductive isolation that exist between the species tested. Apart from stating these results, an attempt will be made to interpret them in terms of the genetical and physiological divergence that has resulted from, or helped to bring about, the recently completed speciation process.

Acheta rubens × NSS

Field-collected specimens of *Acheta rubens* from Viriginia were crossed with field-collected NSS from Quebec (near Montreal) in the spring of 1957, as reported by Bigelow (3)[3]. Only *rubens* ♀♀ × NSS ♂♂ produced hybrid offspring; the reciprocal cross was sterile in this test. The male hybrids developed far more slowly than either the female hybrids or the slow-developing NSS parents. The female hybrids developed at a rate intermediate between that of the NSS and that of the rapidly developing, bivoltine, *rubens* parents. The male hybrids were still immature in January, 1958, after 5 months at temperatures above 75° F and 2 months at 50–60° F. Four of these males were still immature when they died in late April, 1958, after 10 months at temperatures high enough to bring several generations of either *rubens* or NSS to maturity.

Five hybrid males matured during the winter of 1958, the first on January 22. The first hybrid song heard was a distinct trill followed by two chirps (the *rubens* song is a very distinctive trill; that of NSS is a series of shrill chirps). Other hybrid males sang with a series of "trills", each approximately intermediate in length between typical *rubens* trills and typical NSS chirps. Unfortunately no tape recorder was available when these songs could be recorded and consequently the rate of wing stroke, etc. of these hybrid males is not known. The audible differences, however, between *rubens*, hybrid, and NSS songs were readily apparent to all who compared the three sounds.

The number of teeth (Fig. 1) on the file (i.e. stridulatory ridge) was counted on NSS, *rubens*, and hybrid wings and the mean number of teeth, standard

[3]*A. rubens* was then referred to as the "Virginia population", NSS as the "northern spring population".

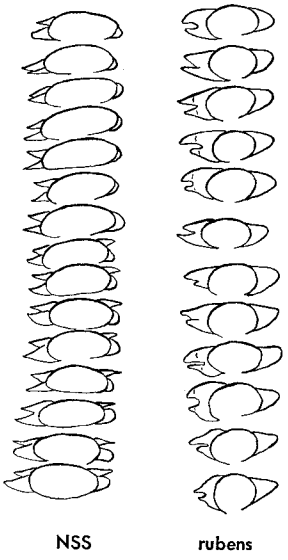

FIG. 1. File teeth of NSS and *A. rubens*, as present near the center of the file, or stridulatory ridge.

deviations, and number of wings examined for each species were as follows:

NSS	138 ± 10.0	($N = 21$)
Hybrids	118 ± 7.5	($N = 9$)
rubens	97 ± 8.3	($N = 36$)

Assuming normal distributions and representative samples, more than 95% of the individual specimens of either *rubens* or NSS can be identified correctly by counting the number of stridulatory teeth. The mid-point between the means for the two species is 117.5, which coincides almost exactly with the mean tooth number of the hybrids (118). The intermediate tooth number in the hybrids suggests that the number of stridulatory teeth is determined by a relatively large number of genes.

The five hybrid males that matured produced no offspring when mated with NSS, *rubens*, or hybrid females.

Hybrid females produced no offspring when backcrossed to *rubens* males. When backcrossed to NSS males, however, a total of 38 nymphs hatched from eggs laid between January 18 and February 1, 1958. Of these 38 nymphs, 5 males and 6 females matured between March 13 and April 14, 1958. On the latter date, only four very small males remained alive (these survived until the end of April and then died). It may be significant that all the F_1 hybrid males, but only half (4/9) of the backcross males developed very slowly (the other five backcross males developed at the same rapid rate as the females). All F_1 hybrid males carried *rubens* X chromosomes; approximately one half of the backcross males carried *rubens*, the other half carried NSS X chromosomes. It is therefore possible that genes affecting developmental rate are carried on the X chromosomes, and that the *rubens* alleles fail to function properly in the presence of certain NSS autosomal genes.

The five backcross males that matured were mated with the six backcross females. Eggs were laid during April, May, and June, 1958, and 58 nymphs (BK_2) hatched from these eggs between May 10 and May 15. Of these nymphs, 13 males and 9 females matured in late June. These were mated with one another, with *rubens* males, *rubens* females, NSS males, and NSS females; the results are shown in Table I.

TABLE I

	BK_2 ♂♂	*rubens* ♂♂	NSS ♂♂
BK_2 ♀♀	One BK_3 nymph	Nil	Nil
rubens ♀♀	One BK_3 nymph	Many nymphs	Nil
NSS ♀♀	Four BK_3 nymphs	Nil	Many nymphs

All six of the BK_3 nymphs died before reaching maturity, and the BK_2 generation itself died out before August 12, 1958. This BK_2 generation was highly variable in size, from extremely small to normal, and they were rather less active than other crickets. The majority died a few days after maturing. Six specimens were observed closely a few hours before their death. They walked slowly with stiff legs when disturbed, gradually weakened, appeared to become partially paralyzed, and finally lay on their backs with their legs waving feebly. Their legs were not curled inwards after death, as are those of most dead crickets, but were extended stiffly outward from the body. At this time these BK_2 crickets were distributed through six different cages, some with *rubens* and some with NSS specimens. The symptoms described above were observed in several of these cages, but only in BK_2 specimens. If the symptoms were due to a disease, *rubens* and NSS specimens must have been distinctly more resistant to this disease. Only BK_2 individuals, of all the hundreds of crickets in the laboratory at the time, displayed the symptoms described. Not all BK_2 individuals died in this way, although the majority died after an abnormally brief life-span. A few survived as adults for $2\frac{1}{2}$ to 3 months.

Throughout the summer of 1958 and 1959 both reciprocal NSS × *rubens* crosses were maintained constantly. Large numbers of specimens were used, including NSS specimens from Viriginia and Michigan as well as from Quebec. Copulation was observed occasionally and eggs were laid (sometimes in large numbers). Hundreds of egg samples were incubated but only two hybrid nymphs hatched: one male and one female. The parentage of these hybrids was NSS ♀ × *rubens* ♂ (the reciprocal of that of the 1957 hybrids). Throughout the periods mentioned above thousands of nymphs were consistently produced by control matings of both species. The hybrid specimens hatched on June 21–22, 1959; the male matured on July 24, the female on July 30. It may be significant that this rapidly developing male carried an NSS *X* chromosome.

The two hybrids were maintained together from July 30 to October 1, 1959. Twelve samples of moist sand were removed at intervals from their cage and incubated. No nymphs hatched.

The song of this male was recorded at 80° F and a portion of it was analyzed by Dr. R. D. Alexander, who found 3–5 (usually 5) wing strokes per chirp, 40–42 wing strokes per second, 230–240 chirps per minute, and 5000–6500 tooth strikes per second. At 85° F NSS produced 25 (range 24–29), and *rubens* 60 wing strokes per second. The mid-point between 25 and 60 is 42.5. The pulse rate (i.e. wing stroke rate) of the hybrid, then, is almost exactly intermediate between those of the two parent species. Rate of wing stroke, therefore, is either determined by the combined action of a number of genes, or else no dominance exists between a single NSS allele on one chromosome and a single *rubens* allele on the other. Perhaps the multifactorial explanation is the more likely; if so this would corroborate the view of Alexander (1) that rate of wing stroke is probably the most significant song characteristic for differentiation between species. If uniformity in rate of wing stroke depends on the presence of an identical set composed of a number of different genes in all individuals of a species, then differences in rate of wing stroke reflect a considerable genotypic difference.

In other song characteristics, however, the hybrid was not intermediate between the parental species. Its song was a series of chirps, for example, rather than a trill. The number of chirps per minute (230–240) lies well within the range of the usual NSS chirp rate (150–240) at the same temperature. Occasionally, at irregular intervals, the chirping rate of the hybrid increased almost to a trill, but these increases did not persist, and the song was never heard to pass into a definite trill, as did the songs of some of the 1957 hybrids. In this 1959 hybrid, then, the tendency to chirp was inherited from the maternal grandfather (NSS) rather than from the father (*rubens*). If the tendency to chirp rather than to trill is determined by autosomal genes, all or most of the NSS alleles are dominant over the corresponding *rubens* alleles. It is possible that some chirp-rate genes are carried on the *X* chromosome, since this would explain both the dominance of chirping in the 1959 hybrid (which carried an NSS *X* chromosome) and the greater tendency toward trilling in the 1957 hybrids (which carried *rubens X* chromosomes). In any case, rate of wing stroke and rate of chirping appear to be determined by different sets of genes.

The number of teeth on the file of this male hybrid could not be counted accurately, but each wing carried at least 125 teeth.

Larger numbers of hybrids should be studied before conclusions can be drawn with full confidence regarding the possibilities discussed above. The difficulty in obtaining hybrids from crosses between these two species shows that the extent of reproductive isolation between them is considerable, but it does not facilitate genetical analysis of interspecific differences in song and developmental rate. Nevertheless, the available evidence suggests (1) that *rubens* genes, affecting developmental rate and carried on the *X* chromosome, do not function well in the presence of NSS autosomal genes, (2) that the song of crickets is a complex character determined by a number of genes, and (3) that certain song components (e.g. rate of wing stroke and rate of chirping) are determined by different sets of genes.

Acheta rubens × *A. assimilis*

Laboratory-reared specimens of *Acheta rubens* (descended from the specimens collected in Virginia in 1957) were crossed with first generation descendants of *A. assimilis*[4] specimens collected in Jamaica in April, 1959.

A total of 101 females and 48 males were used in the two reciprocal crosses, and similar numbers were used in the controls. The crosses were maintained from May 29 to August 13, 1959, and a total of 250 egg samples were incubated. Table II shows the results.

TABLE II

Parentage	No. egg samples incubated	No. samples that produced nymphs	% egg samples that produced nymphs	Total no. nymphs hatched
rubens × *rubens*	46	35	76	Thousands
rubens ♀ × *assimilis* ♂	90	5	6	943
assimilis ♀ × *rubens* ♂	73	15	21	1609
assimilis × *assimilis*	41	22	54	Thousands

Of the 2552 hybrid nymphs hatched, many matured in less than 2 months at 82±2° F. Both males and females derived from the *assimilis* × *rubens* cross (*AR*), and females derived from the reciprocal cross (*RA*), developed at the same rate as nymphs of the two parental species. Males derived from the *rubens* × *assimilis* cross (*RA*), however, developed very slowly. None of these males have matured to date (January, 1960) after more than 5 months at constant temperatures above 80° F. Females hatched at the same time and from the same egg samples matured in September and died after a normal adult life-span of 2 to 3 months. Approximately equal numbers of males and females hatched from each reciprocal cross. Nymphal mortality was high in *RA* males, but 42 are still alive; 38 are in one of the final instars, 4 are in the fourth, fifth, or sixth instars. All these survivors appear to be healthy. In view of the numbers involved, and in view of the fact that not one of the *RA* males developed at a normal rate, it is clear that the cause of this abnormal rate of development is genetic. All *RA* males carried *rubens X* chromosomes; all *AR* males carried *assimilis X* chromosomes; all hybrid females carried one *rubens* and one *assimilis X* chromosome. Rate of development was abnormal only in *RA* males. The abnormality, therefore, is due to genes on the *rubens X* chromosome and these genes are recessive to the corresponding *assimilis* alleles. If it were due to interaction between *assimilis* autosomal genes and *rubens* cytoplasm there should be at least some trace of abnormality in the rate of development of *RA* females, and no such trace is apparent.

All available hybrid specimens were crossed with one another or backcrossed to the parental species. The crosses were maintained from mid-August and mid-December, 1959. The number of adult specimens used and the number of nymphs produced are given in Table III.

[4]*Acheta assimilis* Fabricius (1775), the first name to be applied to an *Acheta* species in America, was based on a type specimen from Jamaica. Since only one species of field cricket of this genus is known to occur in Jamaica, the name *assimilis* is referred to these Jamaican specimens.

TABLE III

Cross	No. of adults used ♀	No. of adults used ♂	No. of nymphs hatched
RA ♀ × *AR* ♂	17	20	2
RA ♀ × *assimilis* ♂	18	12	29
RA ♀ × *rubens* ♂	20	10	17
assimilis ♀ × *AR* ♂	12	17	1427
rubens ♀ × *AR* ♂	14	16	0
AR ♀ × *AR* ♂	32	18	3503
AR ♀ × *assimilis* ♂	19	8	910
AR ♀ × *rubens* ♂	18	12	69
assimilis ♀ × *assimilis* ♂	—	—	Hundreds
rubens ♀ × *rubens* ♂	—	—	Hundreds

The striking difference in the number of progeny produced by these crosses may or may not be significant. The *rubens* adults were rather less vigorous than is normal for this species.

Of the 69 backcross nymphs produced by *AR* females and *rubens* males, 5 females have matured after 52–67 days, 2 females after 80–84 days at $82 \pm 2°$ F, and 1 male matured after 82–89 days at the same temperature. Of the backcross nymphs produced by *AR* females and *assimilis* males, three females have matured after 45–51 days at $82 \pm 2°$ F. All other progeny of these two crosses died in the nymphal stage and the remainder of the backcross and F_2 hybrids are still in the nymphal stage. The mortality of these backcross nymphs appears to have been higher than normal, which may or may not be significant.

Acheta rubens and *A. assimilis* differ distinctly in the color of the hind femur. In *rubens* the hind femur is always dark gray[5] on the outer surface. In most specimens the reddish area at the base of the inner face is continuous on to the base of the outer face, but the entire outer face is distinctly dark. In *assimilis* the color of the hind femur is variable. In many specimens it is pale testaceous with varying degrees of fuscous streaking or mottling, usually along the dorsal edge. In other specimens the testaceous ground color is overlaid with a distinct band or spot of dark chocolate-brown along the dorsal edge, or extending dorsad or ventrad from the ventral or dorsal edge; in still other specimens almost the entire outer face is dark brown. Differences are apparent in the color of the outer face of the two hind femora of certain individual specimens. Even the darkest of *assimilis* hind femora, however, differ from those of *rubens;* the dark brown of *assimilis* and the slate gray of *rubens* femora are readily distinguishable, and the reddish base of *rubens* is never present in *assimilis*.

The hind femora of hybrid specimens are, on the whole, more like those of *assimilis* than like those of *rubens*. The testaceous ground color is apparent in nearly all hybrid specimens, and the dark markings are more like the dark

[5]Alexander (1) refers to the hind femur of *rubens* as "pale". The term is here used in a relative sense, that is, pale in comparison with the black femora of NSS, *pennsylvanicus*, etc. In many *assimilis* specimens the hind femora are far more "pale" than are those of any species treated by Alexander in the paper cited above.

brown of *assimilis* than like the slate gray of *rubens* (possibly because these markings are superimposed on the testaceous ground color). The proportion of specimens with dark hind femora appears to be greater in the hybrids than in *assimilis*, and in the hybrids it is often the base (corresponding to the reddish area in *rubens*) that is testaceous in bicolored femora. This tendency is not apparent in *assimilis*. In some hybrid specimens there is a suggestion of pink at the base of the inner face which may or may not be due to *rubens* genes (the corresponding spot in many *assimilis* specimens tends toward reddish brown), but apart from this, and the basal testaceous areas in bicolored individuals, the reddish basal area of *rubens* is not expressed in the hybrids. The full range of variation in *assimilis*, from almost entirely dark to entirely light, is present also in these F_1 hybrids, about half of which have light hind femora indistinguishable from those of *assimilis*. Differences in the darkness of femoral coloration are shown in Fig. 2.

Although speculation as to the possible genetic basis of hind femoral coloration is premature at this time, it would appear that the *assimilis* condition in general (at least the testaceous ground color and the absence of a basal reddish area) is dominant over the *rubens* condition.

AR males chirped like *assimilis;* none trilled like *rubens*. Since the male parents of these hybrids were all trillers (i.e. *rubens*) the tendency to chirp must have been inherited from their maternal male grandparents through their female parents. *AR* songs are intermediate in wing strokes per second between *rubens* on the one hand and *assimilis* on the other; *assimilis* produces from 90 to 105 wing strokes per second at about 70° F, *rubens* about 60 wing strokes per second at 80° F, and the *AR* males about 75 wing strokes per second at 75° F. Again, the tendency to chirp appears to be determined by genes on the *X* chromosomes.

Acheta assimilis × *A. pennsylvanicus*

On July 31, 1959, three *pennsylvanicus* virgin females were placed in a jar with one *assimilis* male. On August 3 two *pennsylvanicus* males were placed in a jar with one *assimilis* female. The *assimilis* specimens were descended from specimens collected in Jamaica in April, 1959; the *pennsylvanicus* specimens were collected in Quebec, near Montreal, in July 1959 and reared through the final instars to maturity in the laboratory. Egg samples were removed from these jars at intervals until August 17, when both cultures were discarded. No nymphs were produced by the single *assimilis* female and the two *pennsylvanicus* males, but a total of 33 nymphs were produced by the reciprocal cross. The eggs were incubated at a constant 82±2° F and the incubation periods were as follows:

Incubation in days	No. of nymphs hatched
13–15	22
14–16	4
16–19	1
25–27	2
26–31	4

PLATE I

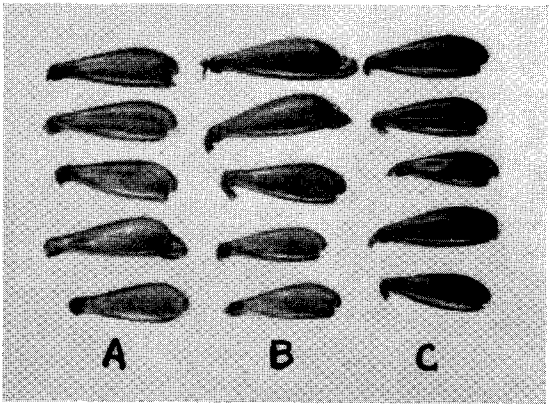

FIG. 2. Hind femora of *Acheta assimilis* (A), *A. rubens* (C), and *assimilis* × *rubens* hybrids (B) showing differences in the darkness of the color of the outer face.

Over 80% of these eggs hatched after less than 19 days of incubation at 82±2° F. This is the incubation period of *assimilis* eggs, which hatch after about 2 weeks at this temperature, or fail to hatch at all. The incubation period of *pennsylvanicus* eggs, however, is usually more than 60 days at this temperature. A few *pennsylvanicus* eggs hatch after about 30 days, but most do not hatch in less than 2 months at 82° F. Of the many thousands of *pennsylvanicus* eggs that have been incubated in this laboratory, not one has hatched after less than 30 days of incubation at 82±2° F.

The incubation period of these hybrid eggs, laid by a *pennsylvanicus* female, was therefore surprisingly brief. The rapid development of the hybrid embryos could not have been due to factors present in (or absent from) *assimilis* yolk or cytoplasm, for both the yolk and cytoplasm of the eggs were derived from a *pennsylvanicus* female. Embryonic diapause in this species appears to be determined, therefore, by the genetic constitution of the embryo and not by cytoplasmic or yolk constituents.

Ten female (three adult, seven immature) and four male (one adult, three immature) hybrid nymphs from this cross were alive in November, 1959. They resembled *pennsylvanicus* in general body color (although the adults were not as jet black as *pennsylvanicus* adults). On one adult female the hind femora were uniformly dark gray, approaching the black condition of *pennsylvanicus*. The other three adults (one ♂, two ♀♀), however, bore a distinct testaceous area near the base and along the ventral edge of the outer face of the hind femur. This condition is, to my knowledge, unknown in *pennsylvanicus*. Of the 10 immature specimens, 9 lacked such a testaceous spot, but it was present in 1 male nymph. On January 9, 1960, four of these females were still alive. All of the four dead females that had not been devoured had distinct testaceous markings on the hind femora; in one case almost the entire hind femur was testaceous. All males were dead at this time, and the testaceous spot was distinct on the femora of the two that had not been devoured (one of these had died very recently after nearly 5 months in the nymphal stage).

Of the 14 specimens alive in November, 4 had testaceous marks on the hind femora, and 10 did not. Of the 10 available specimens on January 9, 8 had testaceous markings and only 2 did not. The testaceous markings, therefore, tend to appear during the later instars.

It is curious that the *assimilis* coloration as a whole, which is expressed in all *assimilis* × *rubens* hybrids, was largely suppressed in these *assimilis* × *pennsylvanicus* hybrids.

Acheta assimilis × NSS

During the summer of 1959 the descendants of *assimilis* specimens collected in Jamaica in April, 1959, were crossed with NSS specimens collected from the field in Quebec in 1959, and with the descendants of NSS specimens collected in North Carolina, Virginia, and Maryland in April, 1958, as shown in Table IV.

TABLE IV

♀	♂	No. of specimens used ♀	No. of specimens used ♂	Set up	Discarded	No. of egg samples incubated	No. of nymphs
NSS (Que.) × *assimilis*		2	2	May 6	June 15	18	0
assimilis × NSS (Que.)		2	4	May 6	Aug. 7	40	0
NSS (Md.) × *assimilis*		1	2	May 6	June 24	24	0
assimilis × NSS (Md.)		1	2	May 14	July 8	14	0
NSS (Va.) × *assimilis*		15	20	May 6	Aug. 11	35	0
assimilis × NSS (Va.)		52	19	May 16	Aug. 11	50	0
NSS (N.C.) × *assimilis*		1	2	May 6	July 22	1	0
assimilis × NSS (N.C.)		1	2	May 11	July 27	20	0
Totals		75	53	May 6	Aug. 11	220	0

Throughout the period indicated above, each of the control matings of *assimilis*, and of all four NSS strains, produced hundreds of nymphs.

Copulation was observed between an *assimilis* female and an NSS (Va.) male on July 10. The spermatophore was effectively transferred, and was carried off by the female. It is regrettable that more time was not spent observing these specimens, but the nature and extent of the work in progress at the time made this impossible. Eggs were laid in all crosses, sometimes in large numbers (occasionally as many as five females were observed ovipositing simultaneously); on the whole, however, females in these crosses laid fewer eggs than did control females. Males other than *assimilis* (i.e. NSS and *rubens* males) often fail to survive through a normal life-span when caged with *assimilis* females. This is perhaps more often the case with specimens caged together in the close confines of a jar (about 5×5 inches) than in cages (24× 16 in.). It has not been noticed in other interspecific matings.

The failure of these NSS × *assimilis* crosses to produce a single nymph shows clearly that factors producing reproductive isolation are effective between the two species, but it does not prove that the incompatibility is absolute (NSS × *rubens* crosses have been sterile for even greater periods of time). It is, of course, impossible to prove a negative.

Acheta pennsylvanicus × NSS

All attempts to obtain NSS × *pennsylvanicus* hybrids in this laboratory have failed. Early crossing experiments involving these two species are discussed by Bigelow (3). These and subsequent experiments will be described by Alexander and Bigelow (2). Large numbers of specimens have been used, and crosses have been maintained over long periods of time (over 12 months). Copulation has been observed, and living sperms have been seen in the spermatheca of an NSS female. This failure to produce offspring shows the presence of very effective isolating factors, but, again, it is impossible to prove a negative.

Acheta rubens × *A. pennsylvanicus*

These two species have not been crossed in this laboratory on a scale comparable with other interspecific mass matings.

Only the *pennsylvanicus* × *assimilis* cross discussed above has involved fewer specimens and less time. Reciprocal *pennsylvanicus* × *rubens* crosses were maintained during the summers of 1957 and 1958, during which time both *pennsylvanicus* and *rubens* controls were producing numerous offspring. No *pennsylvanicus* × *rubens* hybrids were produced, which suggests effective reproductive isolation between the two species (but again without proving absolute incompatibility).

Discussion

The bearing of the foregoing results on the extent of reproductive isolation that exists (or would exist) in the field between these four species must be interpreted with caution. Certain species which would never (or very rarely) interbreed in nature may produce many hybrids in the laboratory, where they have no choice of mates. In crickets of this genus the song of the males is without doubt a potent factor in reducing the number of interspecific matings in the field. In the laboratory, females caged with males of another species were often only inches away from singing males of their own species in a nearby, but inaccessible, cage. Obviously, interspecific matings are more likely to occur under such conditions than they are in nature.

On the other hand, failure of two species to interbreed in the laboratory may bear directly on the extent to which these species can be expected to interbreed in the field. The very factors (absence of choice, etc.) that call for caution in the extrapolation of laboratory interbreeding to field interbreeding may increase the significance of failures to interbreed in the laboratory. If the two reciprocal crosses and the controls were drawn at random from only one population of each species, if the four groups are maintained separately under identical conditions, and if each of the controls produce many offspring while neither of the crosses produce any, then it is safe to conclude that some form of reproductive isolation is present between the two species. If the species will not cross under forced mating conditions such as these it is even less likely that they will do so in the field where a choice of mates is possible.

The failure of *assimilis* × NSS, NSS × *pennsylvanicus*, and *pennsylvanicus* × *rubens* to produce hybrid offspring in the laboratory proves the presence of reproductive barriers more conclusively than the *rubens* × *assimilis*, *rubens* × NSS, and *assimilis* × *pennsylvanicus* hybrids prove the absence of such barriers in nature. Similarly, the infrequent occurrence of *rubens* × NSS hybrids in the laboratory suggests that such hybrids would be even more rare in nature. The production during the summer of 1959 of over 2500 *rubens* × *assimilis*, only 2 *rubens* × NSS, and no *assimilis* × NSS hybrids suggests that isolating factors are least effective between *rubens* and *assimilis* and most effective between *assimilis* and NSS. The production of 33 hybrids

by the first *assimilis* × *pennsylvanicus* cross attempted (only 4 *pennsylvanicus* and 2 *assimilis* specimens were used) may well be fortuitous; future crosses between these species may prove to be sterile. The evidence available at this time, however, suggests that isolating factors between these two species are relatively weak in the laboratory.

Although they do not prove the absence of effective reproductive barriers in the field, interspecific hybrids obtained in the laboratory may be a rich source of information on the genetical similarities and differences between the parental species. Their very existence, for example, proves at least a limited compatibility between the gene complements of the two parent species, and their abnormalities provide clues to genetical differences between these species. When abnormalities of the hybrids involve decreases in vitality or fertility, they provide clues to the particular kinds of divergence that have been most important in the completion of the speciation process.

The *rubens* × *assimilis* hybrids, for example, reveal the presence of an extensive genetical similarity, involving the entire gene complements, between the two parental species. The very arrangements of the genes on the chromosomes is sufficiently similar to permit pairing and the meiotic formation of viable gametes by both sexes of F_1 hybrids. Whether or not the backcross and F_2 hybrids will be equally vigorous and equally fertile remains to be seen, but it is clear from the F_1 that *rubens* and *assimilis* have not diverged far beyond the completion of speciation. Although the evidence of genetic similarity is less complete in the case of *rubens* and NSS, the production of fertile female hybrids by these two species, and the subsequent production by these females of fertile backcross individuals of both sexes, reveals that these two species have also barely completed the speciation process. Although NSS and *assimilis* appear to be intersterile, the close genetical similarity between each of them and *rubens* implies a close similarity with one another. The production of apparently vigorous hybrids by *assimilis* and *pennsylvanicus* implies a genetic similarity between *pennsylvanicus* and *rubens* and, through *rubens*, such a similarity between *pennsylvanicus* and NSS. It is indeed curious that the latter two species, sympatric over much of North America, practically identical in morphology and song, appear to differ most in terms of reproductive compatibility. They have failed, after many attempts, to cross with one another; *pennsylvanicus*, but not NSS, has been crossed with *assimilis*. Although *pennsylvanicus* and *rubens* have not crossed, both have crossed with *assimilis;* although NSS and *assimilis* have not crossed, both have crossed with *rubens;* NSS and *pennsylvanicus* have neither crossed with one another nor with the same third species.

On the one hand, the hybrids show that close genetical similarities exist between all four species, and on the other they show important indications of genetical incompatibility. The abnormally slow developmental rate of all hybrid males that carry *rubens X* chromosomes shows a distinct incompatibility between certain *rubens* genes and certain autosomal genes of both *assimilis* and NSS. Since these *rubens* genes are unable to function normally

in the absence of the corresponding alleles of the other species, certain other genes might also fail to function well when homozygous in F_2 and backcross individuals. The developmental abnormality in the F_1 generation alone provides sufficient grounds for the conclusion that the three species could not, even with panmixia, lose their separate identities in nature. Natural selection favors those individuals which produce the greatest number of viable fertile offspring. The number of fertile offspring from *rubens* × NSS or *rubens* × *assimilis* matings will be, on the average, only three quarters of that derived from infraspecific matings in any one of the three species, which is more than enough to ensure hybrid breakdown. This, of course, depends on whether or not the hybrid males in question are, in fact, sterile. They live, as nymphs, for a long time; four such hybrids from the 1957 NSS × *rubens* cross actually matured, and more than four from the *rubens* × *assimilis* cross may mature. Although none of these hybrids have so far proved themselves to be fertile, some of them may be so. Despite these possibilities, however, such hybrid males are sterile in an evolutionary sense in any environment where the two species undergo seasonal fluctuations in numbers. In Virginia, for example, such males would not mature until long after breeding females have ceased to exist as such in the field. Even if all such hybrid males were to mature simultaneously and all prove to be fertile, they would leave no offspring if there were no females present with whom they could mate. Unless they were able to survive winter conditions they would all be dead before breeding females were again present in the field. Even if a few such males should mature during the following spring or summer and mate successfully with certain females the fertility of this class of males as a whole would be negligible, for it is certain that the vast majority would either mature at the wrong time or die during the winter. Seasonal fluctuations occur even in equable climates like that of Jamaica, where winter does not exist. It is therefore extremely unlikely that *assimilis* and *rubens* would lose their specific identities even if *rubens* were to be introduced into Jamaica in huge numbers.

Similarly, *assimilis* × *pennsylvanicus* hybrids should fail to form an effective bridge for gene interchange between the two species in nature. Even if these hybrids should prove to be fully fertile, and even if all their descendants are equally fertile in the laboratory, it is highly unlikely that the *assimilis* genotype, which has been adjusted to survival in Jamaica, could co-operate for long in nature with the *pennsylvanicus* genotype, which is adjusted to survival in northern regions through periods of sub-zero temperatures.

Developmental rates are of great evolutionary importance in species with distinct breeding seasons and short-lived adults. The developmental rate of each such species is adjusted to the seasonal rhythms of food supply and adverse conditions of the environment in which it lives. Different species are often adjusted in different ways for survival in the same environmental conditions. In Virginia, for example, where *rubens*, NSS, and *pennsylvanicus* are sympatric, *rubens* is bivoltine and overwinters chiefly in the nymphal stage, NSS also overwinters in the nymphal stage but is univoltine, and

pennsylvanicus is also univoltine but overwinters in the egg stage. These characteristics are probably determined, in each species, by the action of a number of different genes, and the combination in hybrid individuals of these two different constellations of genes will tend to make it impossible for either to be expressed effectively. Due to the high adaptive value of the developmental adjustment of each species, intermediate developmental rates will tend to have lower adaptive values (hybrids will be at the wrong stage at the onset of winter for example). Selection against intermediate developmental rates will thus tend to be rigorous. Furthermore, disruption of the finely adjusted sequence of developmental phenomena in either species will tend to produce malformed or otherwise inviable individuals. It is not surprising, therefore, to find that the first distinct sign of genetic incompatibility to appear in these hybrids concerns developmental phenomena. It is also interesting to note that NSS and *pennsylvanicus*, the most similar of the four species in song and morphology, are the most different in developmental characteristics—and also appear to be the most effectively isolated. It is possible that differences in developmental phenomena may reflect divergence toward speciation, or the completion of speciation, more accurately than characteristics such as color, wing length, etc., and this should be borne in mind when attempts are made to solve taxonomic problems at the species level.

The species that produced the most hybrid offspring in the laboratory differed the most in song. This might suggest that song is not important in reproductive isolation. It must be borne in mind, however, that laboratory conditions were unnatural, as outlined above, and also that *rubens* and *assimilis* are allopatric. The *assimilis* chirp is not heard in Virginia and the *rubens* trill is not (to my knowledge) heard in Jamaica. Females of neither species have been conditioned through natural selection to avoid the song of the other species. The failure of *assimilis* and NSS to produce hybrids must be due to some isolating factor other than song, for copulation was observed to occur. It is possible that some factor producing hybrid inviability may exist between these species. The two species with identical songs (NSS and *pennsylvanicus*) appear to be the most effectively isolated, even in laboratory crosses. In nature the difference in their breeding seasons prevents large-scale interbreeding and also inhibits the production of song differences through the action of natural selection; most NSS females will die before *pennsylvanicus* males are mature and most NSS males will be dead before *pennsylvanicus* females mature. Song differences are not required in such cases for the prevention of interbreeding.

Song differences are most likely to be due to natural selection in the case of sympatric species with similar breeding seasons, that is, where males and females of both species are breeding simultaneously in the same habitats. In such cases the risk of a wastage of gametes through outcrossing is high. Males of each species with the most distinctive songs, and females that respond to these songs, will tend to produce larger proportions of succeeding generations; those females which respond to the song of the other species, and

those males whose song is most like that of the other species, will tend to produce fewer viable offspring, and their characteristics will tend to disappear. Obviously, this can happen only when opportunities to outcross are many. It has apparently happened between *rubens* and NSS (which breed in the same fields in the spring) and also between *rubens* and *pennsylvanicus* (which breed in the same fields in the late summer). If the songs of both NSS and *pennsylvanicus* have not changed since their origin from a single ancestral species either the song differences had already evolved between that species and *rubens* before speciation took place, or else the song of *rubens* did all the diverging.

The distinct song differences between *assimilis* and all three continental species have probably come about through random divergence during spatial isolation. Random divergence, then, appears to be as effective as natural selection in the creation of song differences, though it may require longer periods of time.

Of the species tested, the two with the most different songs are allopatric and produced the most hybrids; the two with identical songs are allochronic and appear to be the most effectively isolated. Song could have had little effect either in preventing laboratory interbreeding in the one case or inducing it in the other.

Conclusions

The speciation process has been completed between all four of the species tested, but it has not proceeded far beyond the point where hybrids can no longer form an effective bridge for gene interchange. Certain *rubens* genes, affecting rate of development and present on the *X* chromosome, do not function normally in the presence of either *assimilis* or NSS autosomal genes. These *rubens* genes are recessive to the corresponding *X* chromosome alleles of both *assimilis* and NSS. This single incompatibility is sufficient to ensure hybrid breakdown, and the possibility that numerous other incompatibilities may appear as a result of homozygosity in backcross and F_2 individuals renders hybrid breakdown even more certain. Even those species which produced hybrids in the greatest numbers are, therefore, reproductively isolated in the taxonomic sense, and those species which failed to interbreed in the laboratory are even less likely to interbreed in the field.

The song of male crickets is a complex character made up of several components, not all of which are determined by the same sets of genes. Song divergence may or may not take place in the course of speciation, depending on whether or not recognition factors are required to prevent gamete wastage through outcrossing, and on whether or not random divergence occurs.

Embryonic diapause in *pennsylvanicus* eggs is determined by the genotype of the embryo and not by cytoplasmic or yolk constituents.

Interspecific *Acheta* hybrids offer a promising medium through which to study the microtaxonomy, genetics, physiology, and evolution of *Acheta* species.

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