INHERITANCE OF SONG IN HYBRIDS OF TWO SUBSPECIES OF NEMOBIUS FASCIATUS (ORTHOPTERA).[1]

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In an earlier publication the writer (1) discussed the relationship of three races of *Nemobius fasciatus*, namely: *N. f. socius* Scudder, *N. f. fasciatus* (De Geer) and *N. f. tinnulus* Fulton. The three subspecies have slight morphological and color differences when the average condition of each in respect to certain characters is considered. Some individuals of each race are intermediate in respect to these characters so that it is often impossible to classify a specimen that is not one of a series collected at the same place. *N. f. fasciatus* is the connecting link between the other two races.

PHYSIOLOGICAL CHARACTERS.

In regard to certain physiological characters the three races are more distinct. The song of the male is their most obvious attribute. In this respect *socius* stands apart. Its notes are produced by a rapid vibration of the tegmina as they are brought together. This vibration only becomes apparent when the movement is retarded by low temperature. The notes have a slower frequency and a more buzzing, less musical quality than the other two races. The notes of *fasciatus* and *tinnulus* are single-stroke chirps and their songs differ only in frequency of notes. With *fasciatus* the movement is so rapid at normal summer temperatures as to give the effect of continuous sound but with a noticeable tremolo. When heard singing during cool weather it becomes apparent that each movement of the tegmina produces a single sharp chirp. With *tinnulus* the intermittent character of the song is evident at all times, for the frequency of notes is only about half that of *fasciatus*.

The seasonal history of *socius* in North Carolina differs from that of the other races. It begins to mature in June and has more than one generation annually. No more than two

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generations were obtained in the breeding jars, but both nymphs and adults may be found out of doors in late fall and it seems probable that breeding is continued as long as the weather will permit. *Fasciatus* and *tinnulus* never mature until late summer and nymphs are not found in late fall. In the northern states the time of appearance of adults indicates that no race has more than one generation annually.

In choice of habitat the three races also show marked differences. *Socius* is a grassland race occurring in greatest numbers in moist meadows and marsh borders. In the northern states it is confined to low ground, but in the south it is more widely distributed. *Tinnulus* is a woodland race living in the less densely shaded types of developmental or subclimax woodland or around the borders of climax forest. In respect to habitat also *fasciatus* is intermediate between the other two races. It is associated with grass, but may live in open woodland or under trees where there is enough light to permit a good growth of grass. In the eastern Piedmont region of North Carolina it occurs sparingly in partly shaded places along streams where blue grass and other mesic grasses will grow. In the mountain regions and northern states it is the most common race and occurs in both open and partly shaded places, but is scarce or absent on poorly drained ground where *socius* lives.

In any region there is a marked tendency for each race to occupy its own habitat to the exclusion of the other races. In certain transitional areas like the borders of woodland there is some intermingling of two races. The question arises as to whether they interbreed in such places. Observations in the field have not revealed any individuals that are intermediate in regard to their song, which is always distinctly one type or another.

Socius-tinnulus MATING.

The crossing experiments with *socius* and *tinnulus*, previously reported, show that these two races, which are at opposite extremes in respect to all characters, will frequently mate when confined together. Virgin females were used and the spermathecae examined for evidence of mating. Of seven pairs only two showed empty spermathecae. Of four cases where a male was confined with females of both races only one showed

evidence of mating with the different race, but all mated with their own kind. No offspring were obtained from any of the crosses.

As a result of these studies the writer expressed the belief that the three subspecies are physiologically distinct and that any individual belongs to one of the three, but that morphologically they have not diverged far enough to permit positive classification of all individuals. Further breeding experiments were carried on to test this theory.

CROSSING EXPERIMENTS WITH THREE SUBSPECIES.

During 1930 all of the three races were used in crossing experiments. The race *fasciatus* was obtained from the mountains of North Carolina. Up to that time no *fasciatus* had been found in the Piedmont. All pairs were kept in glass jars with about an inch of fine sandy soil in the bottom. They were fed and watered until the female died.

The following crosses were made:

Females	Males		
	socius	*fasciatus*	*tinnulus*
socius	5 pairs	4 pairs	4 pairs
fasciatus	4 pairs	3 pairs	3 pairs
tinnulus	4 pairs	5 pairs	3 pairs

As a check against possible parthenogenesis several females of each race were kept unmated. After the crickets died, all jars were kept over winter in an unheated insectary and a little water was occasionally added to the soil. During the following spring the soil in all jars was kept moist for a much longer time than would be necessary for fertile eggs to hatch. A large number of nymphs were obtained from all crickets paired with their own race except one pair of *fasciatus*. The female of this pair was found dead on September 29, whereas most of the crickets lived until late October or November. Seven of the eight *fasciatus-tinnulus* crosses gave numerous progeny. One *fasciatus* female paired with a *tinnulus* male gave no progeny. The male of this pair died before October 10 and on October 30 the female was found dead but not decayed. An examination

of the body revealed a few eggs in the ovaries and an empty spermatheca. No nymphs appeared in jars which had contained unmated females or any of the other crosses with one exception. One nymph was found in a jar which had contained a *fasciatus* female paired with a *socius* male. This nymph unfortunately died while still young. There is a possibility that it may have come from a *socius* egg on a small grass clump placed in the jar for food. Grass clumps used were always carefully washed to remove all soil before placing them in the jars, but it is possible that an egg may have been caught in the mass of roots. No race but *socius* was present where the grass was obtained. The later stages of *socius* nymphs can be identified by their markings from the other two races, so the possibility of such a source of error in the *fasciatus-tinnulus* crosses is eliminated.

Among the *socius* paired with their own race was a female from Raleigh with a male from the mountains of North Carolina, where the race is restricted to low ground as in the northern states. A question arises as to whether the wider distribution of *socius* east of the mountains is due to further racial splitting or to a better adaptation of the race to southern environment or to a lack of competition with *fasciatus*. The fertility of the above mentioned pair shows at least a close relationship between the crickets from the two areas.

In the previous year's experiments only two out of seven crosses of *tinnulus* and *socius* showed evidence that the adults had failed to mate. It seems improbable that the failure to obtain progeny in the 1930 crosses involving *socius* could be due entirely to lack of mating. In a desire to obtain as many eggs as possible no females were killed and when found dead they were usually decayed. One female *fasciatus*, which had been paired with a *socius* male for 20 days, was found dead but not decayed. The ovaries were packed with eggs and the spermatheca was empty.

The evidence of this series of experiments seems conclusive that the hybridization of *socius* with either of the other two races would be unlikely to occur in nature. The experiments also show that hybridization of *fasciatus* and *tinnulus* is possible. If the song type of either race were inherited as a dominant character it would be impossible to detect hybrids occurring in nature.

To determine this it was necessary to rear the nymphs to maturity. There was high mortality among them during the hot weather, but five males and several females of the hybrids and several controls of each race survived. The controls sang in the same manner as their male parents, but the song of the hybrids was intermediate between that of the two parent races in frequency of notes. This was apparent even during hot weather when it was impossible to make any close estimate of the chirping rate. Fortunately for the experiment there came a period of cool weather during which the chirping rates could be computed by counting every fourth note for a period of about 10 seconds. This gave a reasonably close estimate for rates up to about 7 per second, but was probably inaccurate for rates higher than that. The records of the hybrid songs are indicated in Fig. 1 by the numeral 1. *Tinnulus* song records are indicated by the letter T, and *fasciatus* by F. Those enclosed in a circle were taken by another method the following year.

In color the F1 hybrids appeared more like *tinnulus*, but the characters of the tegmina were not definitely of either race. Some of the hybrids developed long wings, a dimorphic character common in *fasciatus* but not yet found in *tinnulus*.

F2 GENERATION.

Five pairs of F1 hybrids were mated, one hybrid female was back-crossed with *tinnulus* and two with *fasciatus* males. The jars containing the eggs from these pairs were brought into the laboratory early in the winter in the hope that adults might be obtained during the cool spring weather. Nymphs appeared in January, but they developed slowly and adults were not obtained until June so that there was little prospect of observing the songs under cool weather conditions. In order to get some estimate of the frequency of notes a vibrating interrupter was used. This consists of a long steel spring held by a clamp. By shifting the position of the clamp a wide range of vibrations can be obtained. A needle is attached perpendicularly to one end of the spring. By setting the spring into vibration and allowing the needle point to rub lightly against a thin piece of paper a sound somewhat similar to the chirping of the crickets could be obtained. This was compared to the cricket song and when the sound made by the needle seemed to have the

same frequency as the cricket chirps, the position of the clamp on the spring was recorded. After records were taken at various temperatures the rates were established for all positions of the clamp by recording the vibrations on a kymograph sheet on which second intervals were also recorded. The chief source of error in the method would be in comparing the song with the vibrations of the spring and this would probably not be greater than one note per second. Records of the song of *tinnulus* at 80° F. by this method (T in a circle) are about one

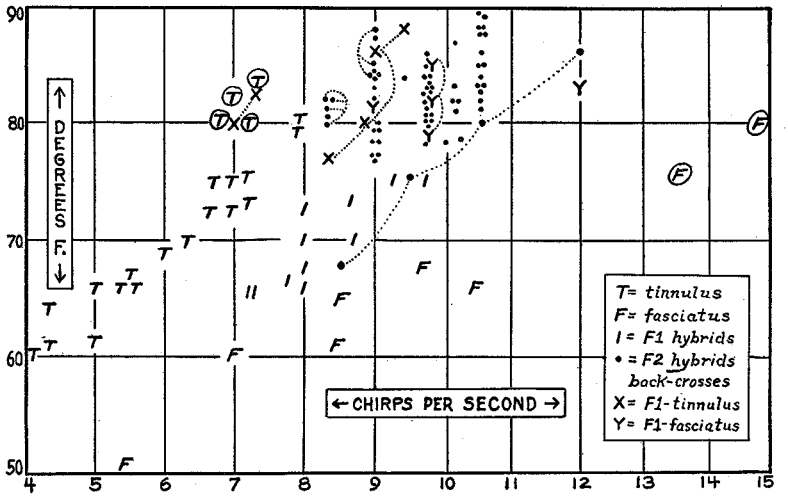

FIG. 1. Chirping rates of *Nemobius fasciatus fasciatus* (F) and *N. fasciatus tinnulus* (T) and their hybrids of the first (1) and second (dots) generation, and progeny of back-crosses of the first generation hybrids with *fasciatus* (Y) and *tinnulus* (X). Records connected by dotted lines are from the same individual.

note per second slower than the estimates made by counting every fourth note. Allowance must be made for inaccuracy in actual chirping rate for records above six notes per second, but the general relative position of the records at any one temperature is more certain. When several crickets are heard singing at one time their relative speeds are apparent even though the determination of the actual rates might be mere guess work.

The records obtained for the F2 generation are shown in Fig. 1 as dots. These were taken from 21 individuals most of which came from one pair of F1 parents. The songs of two

male offspring from F1 and *tinnulus* parents are designated by "X" and three from F1 and *fasciatus* parents by "Y." While these records were taken at higher temperatures than those of F1 songs it can be seen that most of the F2 generation records have an intermediate position also. There is slightly more variation in rate. Some of the F2 songs were consistently more rapid and others consistently slow. The records from a few such individuals are connected on the graph by dotted lines.

One of the offspring from the back-cross with *tinnulus* sang at the same rate as *tinnulus*, but the other was faster. Of the three male offspring from the *fasciatus* back-cross two were like the F2 hybrids and one was faster than the F2 but much slower than *fasciatus*. The two *fasciatus* records above 70° were from specimens collected near Raleigh, while the others, taken the year before, were from the mountain stock used in the experiments.

CONCLUSIONS.

If the intermediate chirping rate of the F1 generation were due to incomplete dominance of a single determiner, the F2 generation should show about one-fourth singing like *tinnulus* and another fourth like *fasciatus*. A few approximated the *tinnulus* song but none the *fasciatus* song. The results are more like what would be expected from the segregation of two or more determiners. The experiments show that F1 hybrids and the majority of the F2 generation would have an intermediate chirping rate which would reveal their presence in the male sex if they occurred in nature. The writer has listened to the songs of these crickets in a number of places where both races were intermingled, both in Iowa and North Carolina, and has never detected a song that seemed to be of intermediate quality. If hybrids occur in nature they must not be of common occurrence. All evidence so far leads one to conclude that the three races of *Nemobius fasciatus* are more distinct physiologically than morphologically, and that they seldom if ever interbreed under natural conditions.

DISCUSSION.

If these insects did not sing it is doubtful whether the existence of *tinnulus* as a subspecies would have been detected.

The typical russet color had been noted in woodland specimens but was considered a variation due to local environmental influence. The species had formerly been divided into two geographic races, *fasciatus* in the north and *socius* in the south. The writer makes no plea for the naming of purely physiological races having no morphological basis. *Nemobius fasciatus* was a border line case in which two races had already been named and the third seemed to have as well defined physical characters as the other two. Physiological races revealed by song differences are not uncommon among the Orthoptera. The races of *Oecanthus niveus* which are purely physiological have been studied by the writer (2). Other cases, which have been detected but not studied experimentally, are found in the genera, *Gryllus*, *Anaxipha*, *Cycloptilum* and *Amblycorypha*.

Since most insects do not sing, one is led to speculate on the number of undiscovered physiological races that may exist among insects, races differing in food habits, seasonal history, ecological distribution and in other characteristics that can be determined only by careful breeding work. The existence of such habit differences could bring about an isolation of races almost as complete as if they were separated by mountain barriers. Barriers are regarded as an important factor in the evolution of species. Whenever diversity of habits in a species reaches the point where adults of one group are separated by time or space from the other, then the practical effect of a barrier is produced.

From an economic standpoint the recognition of the existence of physiological races is sometimes important. For instance, no benefit would be derived from destroying the wild food plants of an insect pest if the species existed as two races, one feeding on the crop and the other on the wild plant. Examples of racial host selection may be found in the works of Thorpe (3) on *Hyponomeuta* and Lathrop and Nickels (4) on *Rhagoletis*. In studies on the resistance of wheat varieties to Hessian fly, Painter (5) has shown that there is evidence for the existence of genetically distinct strains of the fly differing in their ability to infest various wheat varieties. Some physiological characters within a species may have geographical limitations as shown by the work of Hough (6) on the relative resistance to arsenical poisoning by codling moth larvae from Colorado and Virginia, and by Compere and Smith (7) on the resistance of a

California scale insect to a parasite which breeds on the same species of scale in Japan.

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