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### Interspecific Hybridization among Insects with a Report on Crossbreeding Experiments with Stink Bugs<sup>1</sup>

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NOTE: This paper and the three which follow it were presented as a symposium entitled "Genetics in Entomology; Hybridization and Pesticide Resistance" (Dr. H. H. Schwardt, Cornell University, Moderator) at the Eastern Branch Meeting of the Entomological Society of America held in Philadelphia, Pennsylvania, November 16-17, 1953. A paper by Dr. Floyd F. Smith, also presented on this program, was not available in time to be included here.—EDITOR

Hybridization is a subject in which I have been interested for several years. However, being a taxonomist, I have had little time in which to conduct crossing experiments. Data from such work as I have done have piled up in a largely undigested form, and my comments here must be preliminary in nature.

Almost 10 years ago I undertook a revision of the genus *Chlorochroa*,<sup>2</sup> which includes the economically important Say's stink bug (*C. sayi* Stål) and the conchuela (*C. ligata* (Say)). Almost immediately I discovered that in the intermountain region of the Northwest at least three of the species, including the conchuela, break down into a maze of intermediate populations. One of the species is found over most of eastern United States, the conchuela is southwestern but extends north to Canada, and the third species is restricted to Oregon, Washington, and British Columbia. All three occur together in British Columbia, and the first two are found together east of mountains in the northern high plains region. Over much of Montana, Idaho, Utah, and eastern Washington and Oregon populations occur which cannot be assigned to any one of these species and which exhibit characters that clearly suggest hybrid origin involving two or even all three species. This brought my study to a halt, for I couldn't decide whether to deal with the

forms as species, subspecies, local populations, or hybrids.

The possibility that hybrids were involved was suggested by the work of Foot & Strobell (1914), who succeeded in obtaining fertile hybrids between *Euschistus variolarius* (P. de B.) and *servus* (Say), two of the most common stink bugs found in eastern United States. Furthermore, their hybrids presented the same intermediate appearance as did the natural populations of *Chlorochroa* in the Northwest.

I therefore decided to discontinue work on the *Chlorochroa* revision and test the possibility that questionable populations such as those encountered in this genus could be considered of hybrid origin. In this course of action I was influenced by the opinion widely held in taxonomic circles, as late as 8 or 10 years ago, that it was little short of heresy to speak of fertile hybrids between species of animals. There is already considerable literature dealing with interspecific hybrids. Among the earliest well-authenticated records are those concerning butterflies and moths. However, such records were seldom more than notices that a different-looking specimen had been obtained. Few attempts were made to determine whether the hybrids were fertile. Occasionally it was noted that similar specimens had been captured in the field.

About 1920 the first instance of interspecific hybridization was obtained in the genus *Drosophila*. Geneticists were quick

<sup>1</sup> Supported in part by a grant from the Penrose Fund of the American Philosophical Society.

<sup>2</sup> *Chlorochroa* Stål is a homonym, but until certain generic relationships are clarified it seems best to continue using this name rather than to accept a replacement name which might soon be displaced because of generic synonymy.

to utilize hybridization as a tool in their work, and since 1930 much effort has been directed toward such studies in *Drosophila*. Most of these studies have been made by or under the direction of T. Dobzhansky at Columbia University and J. J. Patterson at the University of Texas. This work was brought together and published by Patterson & Stone (1952). Since 1945 many instances of laboratory hybridization between species of mosquitoes and species of Lepidoptera have been reported. In fact, the evidence has now piled up to a point where only the most obstinate or the uninformed continue to hold the view that hybrid sterility is a necessary species criterion.

This raises the question of a species definition, and in my subsequent discussion I use the one originated by geneticists and generally accepted by taxonomists—namely, an actually or potentially interbreeding natural population that is reproductively isolated from other such populations. Since we cannot hope to apply crossbreeding tests to more than a small number of the innumerable forms of life, we all find it necessary to rely on the ability and knowledge of the taxonomist who decides that a form is a species. The taxonomist ordinarily uses structure as the basis for his decision. If a form is distinguished by certain structural characteristics that are not found to intergrade with those of related forms, it is assumed that the population is reproductively isolated and thus qualifies as a species under the geneticist's definition.

All this emphasizes the disjunct nature of species. Yet every taxonomist has repeatedly encountered instances in which his decision to place a form as a species or subspecies was admittedly arbitrary. Judging from the work on *Drosophila*, the geneticist is better off only to the extent that he is able to apply a more discriminating technique to the study of questionable forms. Yet it is clear that often the most careful study of certain forms of *Drosophila* has not allowed definite assignment of species or subspecies status. We are left with the obvious conclusion that life forms grade from freely interbreeding populations, through populations that are reproductively isolated in practice but still potentially capable of crossing and producing some fertile off-

spring, to those that are incapable of interbreeding in any degree.

My purpose here is to consider the possible effect of hybridization on the course of evolution. Hybridization is known to occur at all levels from the intraspecific strain to interspecies, and in some groups even between genera. In the lower levels it is a recognized cause of gene flow and results in the movement of characters through interbreeding populations. The situation is different at the interspecies level, where zoologists generally believe that hybridization has little effect on the course of evolution, and it is with this view that I wish to take issue.

A little over a decade ago Dobzhansky was able to cite only five cases of hybridization in the genus *Drosophila*. Subsequent studies enabled Patterson and Stone to list at least 86 interspecific crosses in the same genus. About 70 of these crosses produced fertile offspring of one or both sexes, and the authors admitted that gene exchange between the crossed species was possible.

At present there are 613 known species of *Drosophila*. They are placed in 9 subgenera, 2 of which are subdivided into species groups—*Lophophora* with 7 and typical *Drosophila* with 22. No crosses between species belonging to different subgenera or different species groups have been obtained. Within species groups the possibilities for obtaining additional crosses are far from exhausted, for only a few of the species have been tested in what approaches all possible combinations. It is also apparent that many of the crosses were successful only after repeated trials.

Not being a geneticist, I should doubtless exercise caution in any expression of views that departs significantly from conclusions based on such a mass of data as those presented by Patterson & Stone (1952). Nevertheless, I do question their statement which reads as follows (page 501):

"In fact the definition of a species as an evolving unit must be based on the concept that the gene systems available to a species as they exist or occur by mutation or recombination are responsible for its further existence and evolution. This does not preclude some hybridization and gene exchange which may benefit a species, but such donations of genes from another form cannot dominate its evolutionary progress."

My reason for questioning this assertion is based largely on their discussion of evolution in the *virilis* group, where they admit the probability that the species *americana* was the result of the natural crossing of *texana* and *novamexicana* (pages 448 and 449):

"The inversions present in *americana* show that this form is a mixture of gene sequences from *texana* and *novamexicana*. It is in this sense a hybrid form, whether or not *texana* and *novamexicana* were species or subspecies at the time their heterozygotes produced the basic configurations of *americana*."

The potential importance of hybridization is again indicated by the evidence cited by Patterson and Stone that in at least three *Drosophila* crosses the males are sterile when backcrossed to females of their parent species but are fertile when mated to hybrid females of the same cross. This strongly suggests the origin of one kind of reproductive isolation.

The insect physiologist Wigglesworth (1953) has recently reported that a German worker obtained hybrids between several hawk moths, and found that the  $F_1$  males were fertile, but that the  $F_1$  females died in the pupal stage. He prevented this mortality by withdrawing all the blood from the female pupae and replacing it with blood from hybrid male pupae. According to Wigglesworth, this is clearly the result of a sex-linked genetic disturbance involving a growth-hormone factor.

Clarke & Sheppard (1953) were able

by a hand-pairing technique to overcome sexual isolation between four species of North American swallowtails and obtain hybrids that were partially fertile only in backcrosses. A steady increase in fertility was obtained with repeated backcrosses. They point out that the members of the "*Machaon* complex" to which these butterflies belong are closely related, and are in fact treated as subspecies by the British Museum. They conclude that these forms must be treated as extreme subspecies rather than valid species, since some of the backcrosses are fertile. In comparison with the studies in the *Drosophila* this is an extremely cautious view, especially since three of the species of swallowtails occur together in northeastern North America and two in the southwestern part of the continent. In fact, the range of one species is completely overlapped by one of the other three.

I have succeeded in obtaining hybrids from reciprocal crosses of the stink bugs *Euschistus servus* and *variolaris*. These two species are found over most of the United States. In most localities where they occur they are often collected in the same sweep of a net. The two species are readily distinguishable in both sexes. Whereas *variolaris* is structurally uniform throughout its range, *servus* breaks up into two subspecies, one being *euschistoides* (Voll.), which until recently has been treated as a distinct species. Typical *servus* is common in southeastern United

Table 1.—Results<sup>1</sup> of breeding experiments with *Euschistus servus* (S) and *E. variolaris* (V) and hybrid strains of these species.

SPECIES OR HYBRID STRAIN	TOTAL FEMALES	MATING DAYS	OBSERVED MATINGS	MATINGS PER MAT- ING DAY	TOTAL EGGS	EGGS PER FEMALE	PER CENT OF FER- TILE EGGS
V	201	4,720	314	0.067	8642	43.0	91.4
S	176	5,003	321	.044	7515	42.7	90.5
S ♀ × V ♂	409	15,409	43	.003	8534	20.9	.4
V ♀ × S ♂	319	13,562	42	.003	6868	21.5	1.7
$F_1$ VS (first series)	7	648	48	.074	2669	381.3	91.0
( $F_1$ VS) ♀ × V ♂	6	263	27	.123	592	98.6	79.1
( $F_1$ VS) ♀ × S ♂	5	234	22	.093	592	118.4	78.9
V ♀ × ( $F_1$ VS) ♂	5	213	20	.094	201	40.2	58.1
S ♀ × ( $F_1$ VS) ♂	7	292	8	.027	253	36.1	64.9
$F_2$ VS	—	—	—	—	894	—	69.2
$F_1$ VS (second series)	5	—	—	—	1760	352.0	85.9
$F_4$ VSS	11	1,690	49	.028	3219	292.6	56.1
$F_1$ VSSS (incomplete)	5	541	7	.013	452	90.4	88.0
( $F_1$ VSSS) ♂ × S ♂ (incomplete)	6	540	9	.017	412	68.7	88.1

<sup>1</sup> The first four lines combine the results of six tests conducted over a period of 2 years, the remaining ten lines are concerned with but one test for each of the hybrid strains.

States, but *euschistoides* extends from coast to coast across the northern part of the country. Through a long belt from Maryland to Kansas the two subspecies of *servus* intergrade, and it is from this belt that my laboratory colonies originated.

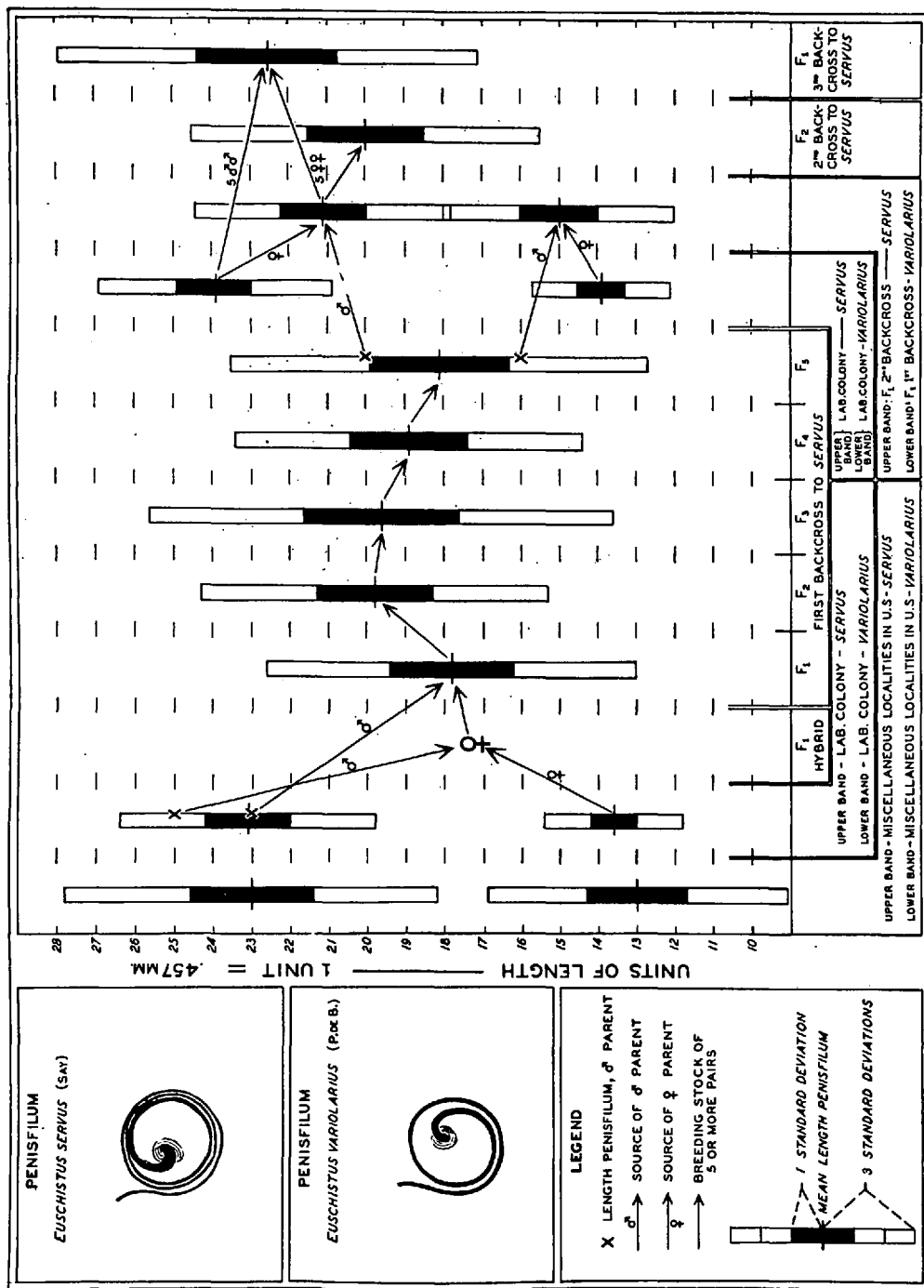
When Foot & Strobell (1914) crossed *variolarius* and *servus*, they carried their hybrids only to the  $F_2$  generation, whereas I have carried populations to the  $F_3$  generation. In addition I have carried a backcrossed line— $\frac{1}{4}$  *variolarius* +  $\frac{3}{4}$  *servus*—to  $F_5$  and then obtained backcrosses providing  $\frac{1}{8}$  *variolarius* +  $\frac{7}{8}$  *servus*,  $\frac{1}{16}$  *variolarius* +  $\frac{15}{16}$  *servus*, and  $\frac{5}{8}$  *variolarius* +  $\frac{3}{8}$  *servus*. In these studies records have been kept which permit comparison of sexual acceptance, egg production, and egg fertility. Reared adults of all lines were preserved in order to learn the effect of hybridization on the characters found useful in distinguishing *variolarius* and *servus*.

The data presented in table 1 indicate the existence of two barriers to successful crossing of *variolarius* and *servus*. First is the barrier of sexual compatibility. In my experiment males and females of the same species were placed together, and at the same time and from same stocks males of one species were placed with females of the other. All females were virgin, and all specimens were of approximately the same age. Twice each day the number of mating pairs was recorded, and any eggs found were removed, counted, and held for hatching. From the number of matings per pair per day it was calculated that the average number of days for one observed mating for each pair was 15 for *variolarius*, 23 for *servus*, and 333 for a female of one species placed with a male of the other. Expressed another way, males will mate with females of their own species 17 times more readily than with females of the other species in the absence of free choice.

The second barrier to crossing of the two species is egg fertility. As shown in table 1, the average fertility of *variolarius* eggs was 91.4 per cent and that of *servus* 90.5 per cent, but eggs laid by *variolarius* females segregated with *servus* males were only 1.7 per cent fertile, and those of *servus* females segregated with *variolarius* males were even less fertile, averaging only 0.4 per cent. Once a sufficient number of individuals were used, these two barriers were broken to the extent that a

few  $F_1$  hybrids were obtained. These hybrids exhibited very great vigor, for 18  $F_1$  females produced on an average 199 eggs apiece, or 4.6 times as many as females of either pure species. The eggs of seven  $F_1$  females mated to  $F_1$  males proved to be 91.0 per cent fertile. Those laid by  $F_1$  females mated to either *variolarius* or *servus* males were about 79 per cent fertile. Eggs produced by *variolarius* females mated to  $F_1$  males were only 58.1 per cent fertile, and those of *servus* females mated to  $F_1$  males 64.9 per cent. There appears to be no sexual barrier between the  $F_1$  hybrids and either pure species. In fact, individuals of either species seem to mate more frequently with  $F_1$  hybrids than with individuals of their own species. However, the 0.027 mating per day for *servus* females with the  $F_1$  hybrid males suggests that the high values for the other combinations involving  $F_1$  hybrids are possibly due to an insufficient sample. The data obtained from a second series of  $F_1$  hybrids, for which only the total number and fertility of eggs were recorded, confirmed the high productivity of  $F_1$  hybrids. Eggs laid by  $F_2$  hybrids were only 69.2 per cent and those laid by  $F_4$   $\frac{1}{4}$  *variolarius* +  $\frac{3}{4}$  *servus* were only 56.1 per cent fertile.  $F_1$  backcrosses from the  $F_5$  generation of this line mated to *servus* produced eggs that were 88 per cent fertile.

Having once produced fertile hybrids between *variolarius* and *servus*, it became possible to study the effect of hybridization on characters commonly used to separate the species. Several external characters, such as color, head shape, and the degree to which the humeral angles are spinose, permit easy recognition of the species. Unfortunately they vary from generation to generation, probably because of environmental factors, so that it is sometimes difficult to evaluate the effect of hybridization. To avoid this difficulty I have used an internal structure of the male genitalia to illustrate the effect of hybridization. This structure is called the penisfilum and consists of a watchspring-like extension of the gonoduct from the aedeagus. The difference between the structure found in *variolarius* and that in *servus* is one of length, as shown in the upper left corner of chart 1. Although its length varies considerably within each species, there is a clear gap between the two which is not



known to be bridged by any specimens collected in the field.

Chart 1 shows the results of measurements taken from males of *variolarius* and *servus* collected at various localities in the United States, from males of my laboratory colonies of both species, and from populations of successive generations of a hybrid strain descendent from one hybrid female backcrossed to *servus*. The measurements from each population are presented as a band showing expected variation based on the standard deviation of the sample studied. Since the samples are not of equal size, the standard errors of the means on which the standard deviations are based are variable, ranging from 0.12 for the parent colony of *variolarius* to 1.5 for the  $F_1$  first backcross to *variolarius*. In no case does the possible error from this source materially affect the trend of variation as shown on the chart. It is clear that the length of the penisfilum in the hybrid strain is intermediate between the lengths characteristic of *variolarius* and *servus*. The range of variability, as shown by the standard deviations, is greater in hybrid populations than in the parent colony of either species. In fact, this variability in the hybrid populations approaches that of the pure species as represented by a sample taken from various localities throughout the United States.

The feature of intermediate inheritance is further illustrated by the backcrosses near the close of the series ending with the  $F_1$  third backcross to *servus*, in which the mean length approaches that of the parent colony of *servus* and the hybrid ancestry is apparent only because of the unusually large variation found in the population. To some degree all the characters in which *variolarius* and *servus* differ show the same kind of intermediate inheritance in hybrid populations as that illustrated by the length of the penisfilum. However, in external appearance  $F_1$  backcross progeny so closely resemble the predominant species that the hybrid ancestry of most specimens would escape the notice of even the most experienced taxonomist.

From the literature on interspecific hybridization, supported by the results of my crossbreeding experiments, I present the following generalizations:

1. Since fertile interspecific hybrid populations can be developed in the lab-

oratory, we can infer that they may also occur in nature. Nature has time, space, and sufficient variability of climatic conditions to permit natural duplication of any such experiment that we can perform in the laboratory. To say that such a phenomenon is restricted to the laboratory is to assert that basic principles governing life in nature are different from those in the laboratory. The problem is one of probability, and although the experimenter cannot create principles in the laboratory he can manipulate probability. Normal probability will account for the frequency of all combinations in a game of cards. However, an accomplished card shark can, through manipulation, obtain desired combinations with much greater frequency than the player who relies upon normal probability. The laboratory experimenter stacks the deck in an analogous manner.

2. If there is no valid reason for doubting the existence of hybrid populations in nature, the taxonomist should consider this possibility when he tries to evaluate an intermediate population. In other words, in addition to the alternatives of species and subspecies, we should add hybrid.

3. In view of the number of successful crosses in *Drosophila*, Culicidae, Lepidoptera, Pentatomidae, we should begin to think of hybridization as a means of breeding new and superior strains of parasites and predators. No doubt some progress in this direction could be made through selection within species, but hybridization can make it possible to introduce a desirable character from one species into another so that an ill-adapted parasite or predator may become a more efficient agent in the control of a pest species. Without question there are serious obstacles to such a program, but most of them can be overcome if there is sufficient knowledge of the behavior and ecological requirements of the species concerned. Plant geneticists have overcome many of the same difficulties. We may admit that animal behavior is more complex than that of plants, but this is no reason for assuming that superior strains of beneficial insects cannot be produced through application of similar techniques. Man has been able to domesticate many animals and modify their structure and behavior to suit his need or fancy. In dogs

it is almost certain that several species were involved.

In recent years we have had a lesson in just how versatile and resourceful our insect enemies can be. They have met one after another of our powerful new insecticides and, after suffering what often appeared to be catastrophic losses, have returned to renew their threats to our com-

fort or economy. There is ample evidence that biological control must hold an important position in our struggle against insect pests, and a breeding program involving the use of hybridization techniques seems to offer great promise as a means of outmaneuvering at least some of them.

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## Hybridization among Mosquitoes and Its Possible Relation to the Problem of Insecticide Resistance

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It is somewhat questionable as to whether a discussion of hybridization among mosquitoes has a place on this symposium, inasmuch as I know of no work that has been done on the inheritance of resistance to insecticides by these insects. My interest in hybridization between populations of mosquitoes has grown out of my earlier taxonomic studies and field experience, when it appeared to me that in order to understand more clearly the biology and the importance of mosquitoes as vectors of disease, it was necessary to think of these insects in terms of populations rather than as sharply divided, morphological species.

This is not the place for a comprehensive review of the work that has been done on mosquito genetics. Recently I have had an opportunity to examine such a review, prepared by Dr. James B. Kitzen. Even though comparatively little work has been done on mosquito genetics, the fact that Dr. Kitzen's manuscript contains over 150 pages shows that in our present discussion we shall not be able to do justice to the subject. As my contribution to this symposium I shall present certain problems involving specia-

tion among the mosquitoes and some of the results we have obtained in our efforts to solve them; secondly, I shall illustrate the mechanisms of inheritance among the mosquitoes; and thirdly, I shall make some suggestions regarding the possible significance of these two aspects of mosquito genetics to the problem of insecticide resistance.

PROBLEMS IN SPECIATION AMONG MOSQUITOES.—It is necessary to refer first to the *Anopheles maculipennis* complex, which has been studied more thoroughly than any other group of mosquitoes. From the earlier work of Hackett, Missiroli, and others we know that what once was thought to be one or two species, is actually a group of at least seven species and subspecies. The cytological studies now being conducted by Dr. G. Frizzi suggests that in turn at least some of these species may be comprised of genetically distinct populations. Frizzi (1951) found that individuals of *Anopheles messeae* from various sampling stations in the Province of Pavia might show a standard

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