

# Perspectives

## Anecdotal, Historical and Critical Commentaries on Genetics

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### **ALFRED HENRY STURTEVANT and Crosses Between *Drosophila melanogaster* and *Drosophila simulans***

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WHEN SEWALL WRIGHT wrote a recommendation for H. J. MULLER in the late 1930s, he described MULLER as perhaps the greatest living geneticist. WRIGHT later expressed this evaluation in conversation on many occasions. But when it came to the relationships among genetics, evolution and systematics, WRIGHT's clear candidate for honors in the MORGAN group was A. H. STURTEVANT.

From his student days on, STURTEVANT had been an avid taxonomist of *Drosophila* and its close relatives. When WRIGHT first met STURTEVANT during the summer of 1912 at Cold Spring Harbor, STURTEVANT spent much of his time collecting wild *Drosophila* (PROVINE 1986). In 1916 STURTEVANT published a paper on North American *Drosophilidae* in which he described 23 new species, including *D. saltans*, *virilis*, *robusta* and *affinis*.

STURTEVANT was also keenly interested in speciation and mechanisms of evolution generally. I have argued elsewhere (PROVINE 1981) that he was probably the author of the first document outlining the ambitious "genetics of natural populations" series carried out by THEODOSIUS DOBZHANSKY and his co-workers.

One of the first published manifestations of STURTEVANT's larger interests in the interactions of genetics, systematics and speciation came with his discovery that *Drosophila melanogaster* was really two species instead of one. STURTEVANT collected *melanogaster* everywhere he went, especially from his own home town of Kushla, Alabama, and brought them back to the laboratory at Columbia. Males from one Kushla stock were bred by Mr. A. M. BROWN (whom I suppose to be a Columbia undergraduate working in the Fly Room) with females from other mutant laboratory stocks. These matings produced only sterile female offspring. STURTEVANT was fascinated by

this unexpected result. He immediately asked his friend, the cytogeneticist C. W. METZ, to send him more wild-trapped *D. melanogaster* from Lakeland, Florida. From these flies, about half of the males produced the same strange results when bred with laboratory females. STURTEVANT gave the flies to C. B. BRIDGES, who quickly discovered a consistent and substantial difference between the male genitalia of the unusual flies and the existing laboratory stocks of *melanogaster*.

STURTEVANT named the new species *Drosophila simulans* Sturtevant and published the description immediately saying, "Since it is evidently a distinct species that has hitherto been overlooked, and since it will certainly be extensively discussed in genetic literature in the future, the following name and description are presented" (STURTEVANT 1919, p. 153).

Why would a new species of *Drosophila* be "extensively discussed"? STURTEVANT's belief was that an understanding of differences between closely related species would give the greatest insight into the process of speciation. Until the crossing of *melanogaster* and *simulans*, no two species of *Drosophila* had ever been known to cross. The beauty of this new species was that it could cross with *melanogaster*, the most studied and best understood species. Here was an opportunity to examine species differences with an exciting set of experimental possibilities. Within less than a year, STURTEVANT prepared for publication a series of three important papers under the title, "Genetic studies on *Drosophila simulans*" (STURTEVANT 1920, 1921a,b).

From STURTEVANT's perspective, *simulans* had the major advantage of producing hybrids with *melanogaster*. But there were also two disadvantages. First, all the hybrids were sterile. No progeny testing beyond the F<sub>1</sub> generation was possible. Second, "since the two parent species are extremely similar and prob-

ably have identical chromosome groups, the data that are to be obtained from the study do not throw as much light as might be wished on many of the problems concerning the nature of the specific differences found in the genus *Drosophila*." Added STURTEVANT, "Nevertheless, the investigation has led to interesting results bearing on such subjects as interspecific sterility, parallel mutations, chromosome maps, and sex determination" (STURTEVANT 1920, p. 488).

By early 1920, STURTEVANT maintained in the laboratory stocks of *simulans* from Florida, New Hampshire, New York, Minnesota, Virginia, Georgia, Alabama, Costa Rica, Panama and Brazil. Why, with the breeding of *melanogaster* in laboratories all over the United States, was *simulans* not discovered and named earlier? STURTEVANT noted that a case of unisexual broods reported by QUACKENBUSH (1910) was almost certainly produced by a cross of *melanogaster* and *simulans*. QUACKENBUSH thought the case was important because it might shed light upon the problem of sex determination, but he guessed nothing about another species being involved. (Incidentally, QUACKENBUSH did this work at Columbia when STURTEVANT was an undergraduate.) My own guess is that such matings between *melanogaster* and *simulans* had been observed but attributed to bad media, X-linked lethals, or something of the sort. STURTEVANT always valued exceptions and the unexpected.

The three papers, "Genetic studies on *Drosophila simulans*," bore the subtitles, "I. Introduction. Hybrids with *Drosophila melanogaster*," "II. Sex-linked group of genes" and "III. Autosomal genes. General discussion." The easiest cross was between *melanogaster* females and *simulans* males. Only sterile female offspring were produced, bearing the expected female sex-linked characters. STURTEVANT also crossed XXY *melanogaster* females with *simulans* males, producing the usual daughters but also sons with sex-linked genes from the father (Y egg from mother, fertilized by X sperm). The reciprocal mating of *simulans* females with *melanogaster* males produced males with the usual sex-linked characters, and a very occasional female (which STURTEVANT could not explain). Mating XXY *simulans* females with *melanogaster* males produced regular sons and females with sex-linked characters only from their mothers (XX eggs and Y sperm).

When STURTEVANT compared these matings, he discovered that identical chromosome complements (e.g., in reciprocal F<sub>1</sub> females) fared differently depending upon the cytoplasm of the mother. Thus he concluded that species differences pointed clearly to cytoplasmic differences as well as chromosomal genes. This was a very important observation. In addition, STURTEVANT observed that sexual selection was a powerful factor in preventing crossing between *melanogaster* and *simulans* in nature. Females were far

more likely to mate with males of their own species (although males were ready to mate with females of either species). "Sexual selection, then, is one means whereby the two species are kept from crossing." But, STURTEVANT added, this barrier would "probably be ineffective if fertile hybrids were produced when cross-mating does occur" (STURTEVANT 1920, p. 499).

Complete sterility of the hybrids was a sadness to STURTEVANT. "It had been hoped, had the hybrids been fertile, that the genetic make-up of *simulans* could be studied through the hybrids. This hope disappeared when the hybrids were found to be sterile, so the problem had to be attacked in another way, — namely, by studying the genetics of pure *simulans*" (STURTEVANT 1921a, p. 43). STURTEVANT did a neat little trick here. He simply examined hybrid females with chromosome complements from both *melanogaster* and *simulans*. If two similar X-linked recessive mutations from the two species produced a recessive phenotype, they were allelic, and if the dominant phenotype appeared, then the two mutations were not allelic. Of six X-linked mutations tested, five were allelic in the two species. Clearly, the two species were very closely related. Oh, how STURTEVANT wanted to see the F<sub>2</sub> generation of the cross between *simulans* and *melanogaster*!

In the third paper of the series, STURTEVANT used the same technique as in the previous paper but focused upon the autosomal genes of *simulans* and their relationships with the autosomal genes of *melanogaster* and other species of *Drosophila*. Two of four third-chromosome recessive mutations in *simulans* were allelic with recessives in *melanogaster*; none of four recessive mutations on the second chromosome, however, had corresponding alleles in *melanogaster*. The linkage groups were very similar (but he had no mutations on the fourth chromosome to work with). From these data and cytological examination, STURTEVANT concluded that the chromosome groups were in fact physically similar. In each of the linkage groups, the order of the mutations appeared to be the same (this conclusion would soon change).

In this set of three papers, STURTEVANT had proved that two closely related species had newly recurring mutations that were allelic and thus probably identical. He claimed that this was the first demonstration of parallel mutations in two distinct species (STURTEVANT 1921b, p. 205–206) and I see no historical evidence to the contrary.

Clearly *simulans* and *melanogaster* were two species. Hybrids between them always had only rudimentary sex organs and no hybrids were fertile. Where were the hereditary differences that made them into good species? One place where such differences definitely existed, according to STURTEVANT's evidence, was the cytoplasm. He suggested another extremely interest-

ing possibility in a summary section titled "Complemental Genes" (STURTEVANT 1921b, p. 200–201). Pointing to "the extraordinary variability that has been observed in the  $F_2$  generation of many species crosses," STURTEVANT argued that many of the genes in a species had their greatest phenotypic effect when in the presence of the genes of another closely related species. "Such genes are perhaps to be thought of as having arisen by mutation and having been perpetuated through chance. Since they do not produce any significant effect in the genetic complex in which they arose, they are not eliminated by natural selection, and some of them will sooner or later happen to be incorporated into the race" (STURTEVANT 1921b, p. 200).

Random genetic drift (of course STURTEVANT did not have the name, which was yet to be invented) was perhaps a key to understanding species differences! This was precisely the argument given by SEWALL WRIGHT in the years 1929–1932, but I for one had never appreciated STURTEVANT's suggestion in this paper. (WRIGHT's copy of the paper is checked on the front as having been read; there are no marginal checks or comments.)

While this paper was in press, STURTEVANT discovered that one of his conclusions required revision. He had found two more mutant genes on the third chromosome of *simulans* that were allelic with two known genes in *melanogaster*. Crossover frequencies showed clearly that identical loci were not in the same sequence. STURTEVANT reviewed the evidence for a translocation, the explanation favored by BRIDGES and WEINSTEIN, then offered his own suggestion: "the simple inversion of a section of a normal chromosome."

Such an accident seems not unlikely to occur at the stage of crossing over. If we suppose a chromosome to occasionally have a "buckle" at a crossing over point, it is conceivable that crossing over might be followed by fusion of the broken ends in such a way as to bring about an inversion of a section of the chromosome (STURTEVANT 1921c, p. 236).

So inversions were also possible factors in species differences. First-generation crosses between *simulans* and *melanogaster*, under STURTEVANT's analytical eye, yielded a cascade of important insights into species differences.

STURTEVANT continued his work on *simulans* during the 1920s. A brief report of this research can be found in each *Year Book of the Carnegie Institution of Washington*. STURTEVANT and PLUNKETT (1926) published further evidence indicating that the third chromosomes in *simulans* and *melanogaster* differed by an inversion. Three years later, STURTEVANT (1929) published his major summary paper on the genetics of *Drosophila simulans*. He described newly discovered

mutations, but little of interpretive interest had changed in the meantime.

Because he had done just about all that he considered interesting and possible on the crossing of *simulans* and *melanogaster*, STURTEVANT turned his attention to other problems. As he had stated earlier, STURTEVANT wanted the hybrids to be fertile so that progeny testing for later generations would be possible. Thus, when he wrote his famous three little "Essays on Evolution" (STURTEVANT 1937, 1938a,b), he neither mentioned nor cited any of his work on the crossing of *simulans* and *melanogaster*, not even in the last one, subtitled "On the origin of interspecific sterility." Instead, he talked about the differences between races A and B of *Drosophila pseudoobscura*, arguing that interspecific sterility was probably a product of the occurrences of inversions and small translocations between populations, thus introducing a loss of fertility in hybrids. Under natural selection (on the argument of FISHER), any tendency not to blunder into producing sterile hybrids by cross-mating would be amplified by natural selection, leading to complete isolation of the species.

As I have argued earlier (PROVINE 1981), STURTEVANT probably gave up the *simulans-melanogaster* crossing because the hybrids were completely sterile. LANCEFIELD's discovery of the two races of *D. pseudoobscura*, which could produce some  $F_2$  and backcross hybrid offspring, offered much greater possibility for genetic analysis than with the *simulans-melanogaster* crosses.

But STURTEVANT also believed that something crucial was missing from the *pseudoobscura* case. He did not believe the two races A and B were different species. There were no certain taxonomic markers, such as the male genitalia differences between *melanogaster* and *simulans*. Thus, for STURTEVANT, elucidating the differences between these two races was not as significant as doing the same for two different species. When DOBZHANSKY and EPLING (1944) renamed the races A and B as *D. pseudoobscura* and *D. persimilis*, STURTEVANT (1944) sharply disagreed with their classification. Whether or not DOBZHANSKY and EPLING were justified in their classification, STURTEVANT was right that finding out more about the differences between *simulans* and *melanogaster* was crucially important to understanding more about speciation. Yet it was also true that STURTEVANT had reached an impasse because of the total sterility of the hybrids.

This is the end of the story of STURTEVANT's work on the crossing of *simulans* and *melanogaster*, but it is really only the beginning of what has become a very lively and complicated story that continues to unfold at the present day. Only the barest outline is possible here.

Soon after STURTEVANT published his three papers on the crosses between *melanogaster* and *simulans* in 1921, the Swedish geneticist GERT BONNIER, who was keenly interested in the whole issue of speciation in *Drosophila*, began to work on the *melanogaster-simulans* hybrids. He believed that the differences between the two species could not be fully understood by STURTEVANT's genetical approach:

As it appears to me necessary for the purpose of gaining a deeper insight into the question, investigations of a physiological, microchemical and anatomical nature on the relations between the two species . . . may be made . . . I hope that these studies will only be precursory to others of the same nature . . . The question of the interspecific relations between *melanogaster* and *simulans* appears to me of such import for a great part of the problem of heredity research, that I believe it to be worth while to attack this question from several different points of view (BONNIER 1924, p. 58).

BONNIER's histological study of the hybrids, based upon only a few individuals, clearly did not impress STURTEVANT (1929, p. 9), but did open this general approach to the study of the cross and led to a series of investigations in the 1930s.

Primary among these were the studies of JULIUS KERKIS of the Laboratory of Genetics, USSR Academy of Sciences in Leningrad. During the 1930s, KERKIS conducted a series of investigations of the influence of temperature on the hybrids (KERKIS 1933a), development of gonads in the hybrids (KERKIS 1933b) and chromosome conjugation in the hybrids using the giant salivary chromosomes (KERKIS 1936, 1937). Independently, KLAUS PÄTAU, of the Max Planck Institute in Berlin-Dahlem (later of the University of Wisconsin), reached conclusions similar to those of KERKIS' 1936 paper (PÄTAU 1935).

In 1933, JACK SCHULTZ and THEODOSIUS DOBZHANSKY published their results from crossing *D. simulans* males with *D. melanogaster* triploid females. They saw no way at the time to produce recombinants using this cross.

Enter MULLER again. MULLER was always trying to find techniques that could accomplish the apparently impossible. What STURTEVANT wanted was the recombinant types that normally appear only when the hybrids are fertile. MULLER and PONTECORVO (1940a,b; 1942) and PONTECORVO (1943a,b) figured out a way to produce these genotypes by crossing triploid *melanogaster* females (which often produce gametes with extra chromosomes) with irradiated *simulans* males (which sometimes produce sperms with missing chromosomes). This technique enabled them to produce, for example, a fly with a third chromosome from *simulans* and one from *melanogaster*, but with all other chromosomes from *melanogaster*. MULLER and PONTECORVO concluded that all genetic factors causing

hybrid sterility between *simulans* and *melanogaster* were on the chromosomes. They also found that the sterility factors were found throughout the genome; moreover, the factors were highly interactive. They concluded that even these closely related species differed by a large number of genes that affected fertility.

The final conclusion of MULLER and PONTECORVO (1942) is worth quoting in full, as PATTERSON and STONE (1952, p. 388) already noted and quoted:

The fact that even these minor chromosomes exhibit so many gene differences indicates that the reaction systems producing the similar phenotypes of apparently closely related species may be highly divergent. Hybrid sterility is but one expression of this cryptic divergence, which need not in itself have had a selective value.

This conclusion is notable not only for its challenge to future students of speciation (species differences being very complex genetically) but also because it raises again the thesis that species differences may have come about by random genetic drift of genes that have little or no selective value.

The status of hybrids in the genus *Drosophila* was covered in detail by PATTERSON and STONE (1952, Chapter 9). The hope of understanding the *simulans-melanogaster* cross has never died. A combination of molecular biology and new discoveries of genes that rescue the classes of hybrids that always died in STURTEVANT's crosses has renewed interest in this historically important species cross.

In 1979 WATANABE discovered a gene in *simulans* that rescued the lethal hybrids, and in 1987 HUTTER and ASHBURNER discovered a gene in *melanogaster* that accomplished the same lethal hybrid rescue (see also HUTTER, ROOTE and ASHBURNER 1990 for a more detailed account of this gene). WATANABE and HUTTER and ASHBURNER suggest that, because a single gene is found to rescue the lethal hybrids, studying hybrid rescue loci may elucidate the genetic basis of speciation. MULLER's belief was that a great many genetic factors were involved with speciation of *D. melanogaster* and *D. simulans*, and that studying one locus would reveal rather little.

COYNE, who has also been working on the genetic basis of sterility between closely related species of *Drosophila* (COYNE 1985; COYNE and ORR 1989), including *simulans-melanogaster* hybrids, thinks that MULLER's thesis deserves very careful consideration before being rejected (J. A. COYNE, personal communication). His hunch stems from the focus of MULLER and PONTECORVO upon the sterility of the hybrids rather than upon the rescue of lethal classes of hybrids. If the genes for lethal hybrid rescue had been found in 1940, I suspect that MULLER would not have changed his opinion, based as it was upon recom-

binants rather than flies hybrid for each chromosome pair. Time will tell the outcome of this important difference of opinion about genetic differences and speciation.

STURTEVANT believed in 1921 that understanding the genetic differences between *melanogaster* and *simulans* would not only elucidate the genetic basis of sterility but also yield insights into mechanisms of speciation. For 70 years his belief has stimulated a steady flow of important research that still exhibits great vitality.

An excellent recent analysis and overview of the *simulans-melanogaster* hybrids, complete with detailed bibliography, can be found in ASHBURNER (1989, pp. 1178–1190).

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