

Population Structure in the *Culex pipiens* Complex of Mosquitos*

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Mosquitos of the Culex pipiens complex offer unique opportunities for study of the mechanisms that separate populations and of the possible advantages of the blood-feeding habit. C. pipiens is a polytypic species in which there are at least three major adaptations to environment. The tropical quinquefasciatus (fatigans) is a generalized blood-feeder dependent on the year-round availability of a variety of hosts. The obligate blood-feeding pipiens form of the temperate zone appears to be adapted to feeding upon nesting birds. The autogenous molestus form is essentially non-blood-feeding. Additional variants are present in different parts of the world.

The variety of devices that serve to separate these different populations pose difficult problems for the taxonomist.

Mosquitos of the *Culex pipiens* complex offer unique opportunities for study in two areas of basic biological interest. One relates to mechanisms that separate populations and the other to possible advantages of the blood-feeding habit. Genetically distinct autogenous and anautogenous biotypes frequently coexist in regions where there are prolonged periods of cold weather. They differ in their ability to produce eggs without feeding upon vertebrate blood and may, in this sense, be characterized as facultative and obligate parasites, respectively. They also differ in their method of overwintering.

This difference in overwintering has been stressed in published reports of the distribution and abundance of the biotypes (Laven, 1951; Roubaud, 1933). Anautogenous populations are thought to survive the winter readily, whereas autogenous populations are less successful in overwintering because they require sheltered breeding sites for winter survival. During the summer months, however, autogenous mosquitos become more numerous as a result of

their ability to reproduce in the absence of vertebrate hosts. It follows from this that hybridization of the biotypes may be limited because the ability to hibernate is a recessive character (Vinogradova, 1961). Such non-hibernating hybrids would presumably appear toward the end of the breeding season and would be eliminated if exposed to cold weather. Autogeny is semi-dominant (Spielman, 1957) and, therefore, only a portion of a hybrid population could colonize sheltered breeding sites.

In addition, it is now suggested that the relative abundance of the autogenous and anautogenous biotypes may be a function of the adaptive advantage conferred by blood-feeding. The extent of mutual gene exchange would, in large part, be determined by the fitness of hybrids for either facultative or obligate blood-feeding. In the following discussion, this concept will be developed and the structure of *C. pipiens* populations reviewed.

RELATIVE FECUNDITY

Females of the autogenous and anautogenous biotypes each produce a characteristic number of eggs. Anautogenous females are more fecund and may produce more than 400 eggs after feeding on a chicken (Christophers, 1945; Tate & Vincent, 1936). The mean number of eggs produced in this manner by females of an anautogenous strain isolated in Boston was 243. In contrast, females of

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an autogenous strain from the same location produced a mean of 68 eggs without food of any kind. It is difficult to induce autogenous females to take a meal of blood before they have deposited their first autogenous egg clutch, and blood-feeding by nulliparous autogenous females is probably a rare event in nature (Tate & Vincent, 1936). Although subsequent ovarian cycles require blood-meal stimulation, the egg clutches then do not appear to be significantly larger than the first autogenous clutch (Christophers, 1951; Dobrotworsky, 1954; Kal'chenko, 1962). Females of the Boston autogenous strain, after feeding on a chicken, developed a mean of 83 eggs, slightly more than were developed in the prior autogenous clutch. In any case, multiparous females in natural populations in Boston were rare and fecundity is thus primarily a function of the first egg clutch. It is evident, therefore, that the obligate blood-feeder enjoys a potential advantage over the facultative. In the presence of suitable hosts, the former may reproduce at a greater rate.

Although autogenous females are potentially less fecund than anautogenous females, they possess an important advantage in that they are capable of reproduction when deprived of food. Virtually all inbred autogenous females deposit eggs without feeding (Spielman, 1957, 1964). In contrast, only one-third of the females resulting from the hybridization of North American autogenous and anautogenous strains produce eggs autogenously. Another third initiate autogenous development but do not develop mature eggs and the remaining third are phenotypically anautogenous. The egg-laying potential of a hybrid population is, presumably, less than that of inbred autogenous or anautogenous populations, owing to the fact that the presence of partially formed ova will interfere with the synchronous development of a maximum number of ovarian follicles.

REPRODUCTIVE ISOLATION

Hybrid populations appear to be less well adapted than inbred autogenous or anautogenous populations to natural conditions. They appear not to overwinter effectively and their fecundity is reduced. This suggests that autogenous and anautogenous populations may become reproductively isolated in nature. Indeed, hybridization appears to be rare among natural populations in Boston (Spielman, 1964). Although 20% of the total population sampled was autogenous, less than 3% of captured females

produced mixed progeny and were presumed to be hybrids. In the event of panmixia, hybrids would have been far more numerous. Confirmatory evidence was derived from another study that will be presented in detail elsewhere. Larvae that had been captured in several breeding sites were reared and the resulting adults were tested for autogeny. Here, incomplete autogenous development served as the criterion of heterozygosity. Less than 2% of those females initiating autogenous development failed to produce mature eggs. The incomplete expression of autogeny in inbred colonies approaches this frequency. It was concluded, therefore, that these populations show relative isolation in nature but that there may be limited gene exchange between them.

Autogenous and anautogenous populations from eastern North America hybridize readily when reared in the laboratory (Rozeboom, 1958; Spielman, 1957) and reproductive isolation of sympatric populations must, therefore, require the action of effective mating barriers. Differential sexual activity was interpreted as being a potential mating barrier by Rozeboom & Gilford (1954), and Dobrotworsky (1955) suggested that differences in flight activity may be the basis of this differential. Autogenous *C. pipiens* are generally stenogamic, mating with a minimum of flight activity (Roubaud, 1933). This behavioural factor may therefore serve to isolate autogenous and anautogenous populations in nature. Anautogenous mating may occur solely after prolonged flight, whereas autogenous mating may take place close to the point of emergence of the adults from the breeding site.

Autogenous and anautogenous *C. pipiens* have frequently been taken in the same collections (e.g., Dobrotworsky, 1955; Knight & Abdel-Malek, 1951). Similarly mixed collections were commonly observed in the Boston studies, and reproductive isolation, therefore, could not be attributed to geographic or seasonal separation of these populations.

RELATIVE ABUNDANCE

The specific biological requirements of the two populations serve to separate them ecologically. The anautogenous form cannot reproduce without access to suitable hosts and the prime requirement of an anautogenous breeding site is that it is freely accessible to females that have taken a meal of vertebrate blood. Such exposed breeding sites as open

ditches and rain barrels are characteristic of this form, whereas the autogenous form is commonly found in subways and covered wells (e.g., Kamura, 1959; Shute, 1951). Chemical and physical properties of the larval environment have been studied as possible causes of this differential. Although high organic content is frequently correlated with autogenous breeding, this is not invariably the case (Knight & Abdel-Malek, 1951). Other factors, such as pH and the presence of dissolved salts, are less strongly correlated (Kamura, 1959) and are probably not determining factors.

That anautogenous breeding sites may be characterized by their ready accessibility to gravid mosquitos is suggested by the following unpublished observations. Two adjacent, underground breeding sites in Boston were studied over a three-year period. Both were initially colonized by anautogenous mosquitos during each breeding season. One site remained predominantly anautogenous, while the other became progressively autogenous as each season progressed. The sites differed in that the former communicated readily with the surrounding environment, whereas the autogenous site was virtually enclosed. Autogenous populations may increase in density in complete isolation from vertebrate hosts. Restricted egress from the breeding site should selectively increase autogenous population growth, and autogenous breeding sites appear to be characterized by this condition.

The high egg-laying potential of the anautogenous biotype is realized solely after feeding upon avian hosts (Woke, 1937). In contrast to the average of 243 eggs produced after an avian blood-meal, a mean of 111 eggs was produced by females of a Boston strain after feeding on man, only slightly more than were produced by autogenous females without blood-feeding. Laboratory colonies of typical *C. pipiens* are commonly maintained with avian hosts. More females will feed upon birds and more eggs will be produced. In nature, these mosquitos show a strong preference for avian hosts (Hayes, 1961). It is evident, therefore, that man-biting reduces a female's reproductive potential and that host preferences tend to compensate for this factor in nature.

In Boston, *C. pipiens* females rarely feed on man. *C. salinarius* is frequently confused with *C. pipiens* and, although far less numerous than *C. pipiens*, is responsible for most of the annoyance due to *Culex* mosquitos in this area. Although the autogenous form is frequently described as being anthropophilic (e.g., Roubaud, 1933; Shute, 1951), man-

biting rarely occurred when man was exposed to known autogenous populations in Boston (Spielman, 1964). These autogenous mosquitos appeared to be essentially non-blood-feeding in nature. Blood-gorged females taken from a mixed autogenous-anautogenous population were exclusively anautogenous. Although human hosts were nearby, birds comprised the source of the blood-meals of most of the captured mosquitos.

Nesting birds or nestlings appeared to be the prime blood source of *C. pipiens* in Boston. There was a preponderance of blood-gorged females during the nesting season, but unengorged non-gravid females became more numerous thereafter. Anautogenous populations began to develop early in June, after the bird-nesting season was under way, and abruptly declined during August, once the nests had been abandoned. Autogenous populations continued to increase in density until September.

The relatively large egg-laying potential of females of the anautogenous biotype suggests that they may proliferate rapidly. Furthermore, their developmental cycle is shorter than that of autogenous mosquitos (Lang, 1963). That anautogenous populations in nature develop the more rapidly was confirmed in observations on Boston populations, their rate of increase being approximately one-third greater than that of autogenous mosquitos. However, various environmental factors may influence this developmental differential. Since the two populations tend to develop in breeding sites of different types, local conditions of food supply or water temperature may favour one or the other form.

The range of the autogenous biotype is strongly influenced by its inability to hibernate (Roubaud, 1933; Vinogradova, 1961). Anautogenous females develop a large fat body with the onset of winter and are thereby enabled to survive long periods of inactivity in an environment of suitable temperature. Although near-freezing temperatures delay ovarian development in autogenous females (Spielman, 1957), such insects do not appear to be capable of hibernation. All hibernating females captured in Boston were anautogenous. The range of the autogenous biotype is, therefore, restricted to locations in which uninterrupted breeding is possible throughout the year. In general, such breeding areas are characteristic of urban environments and the autogenous form has been characterized as an "urban biotype". Anautogenous populations have no such restriction in range.

DESCRIPTION OF ADDITIONAL POPULATIONS

In tropical regions, a third biotype (*quinquefasciatus*)¹ is present that is morphologically distinct from the previously mentioned temperate-zone forms. The anautogenous temperate form will be designated "*pipiens*" and the autogenous form "*molestus*" in the discussion that follows. A broad zone of intergradation joins the ranges of *quinquefasciatus* with those of *pipiens* and *molestus* (Barr, 1957; Mattingly, 1951). In Australia, however, introduced *molestus* populations may be sympatric with *quinquefasciatus* (Drummond, 1951).

Females of the *quinquefasciatus* form feed upon man relatively frequently in nature and are acknowledged to be important vectors of human disease (Mattingly, 1963). In the laboratory they appear to be less host-specific than the anautogenous *pipiens* form, feeding nearly as readily upon guinea-pigs or men as upon chickens. This low order of specificity is correlated with a lesser fecundity (Christophers, 1951). The mean number of eggs produced in the laboratory by females of a strain derived from Texas was 131. This suggests that a low order of specificity in parasitic mosquitos may be correlated with a reduced reproductive potential.

Although *quinquefasciatus* populations may be reproductively isolated from typical *pipiens* populations in certain regions of overlap, in others apparent morphological hybrids are numerous (Barr, 1957). Individual egg-rafts of a series of these apparent hybrids contain morphologically intermediate specimens in association with typical *pipiens* or *quinquefasciatus* (McMillan, 1958). This indicates that, in at least a portion of the zone of overlap, the populations hybridize.

The seasonal distribution of *pipiens* and *quinquefasciatus* populations in regions of overlap suggests that the high reproductive potential of the former enables it to increase more rapidly than the latter. In Kansas, *quinquefasciatus* populations do not become numerous until the end of the summer, *pipiens* populations predominating earlier in the season (McMillan, 1958).

The *C. p. pallens* variety of Japan constitutes a fourth major component of the *C. pipiens* complex. It is a homogeneous group of organisms, morphologically intermediate between the *pipiens* and *quinquefasciatus* forms (Sasa et al., 1963). Although it may have arisen through hybridization, its homo-

geneity implies that it forms stable populations. Certain *pallens* and *quinquefasciatus* populations from the Japanese islands differ in their temperature-tolerance and are reproductively incompatible in laboratory culture (Sasa et al., 1963).

Additional varieties have been reported from various parts of the world (Dobrotworsky, 1955; Laird, 1961; Rioux & Pech, 1959). Two Australian forms, *globocoxitus* and *australicus*, are morphologically distinct from other members of the *C. pipiens* complex and are reproductively isolated in nature (Dobrotworsky, 1955). A variety of isolating mechanisms appears to separate these populations from each other and from the more widely distributed forms. These include, among others, ecological separation, mechanical incompatibility and preferential mating. When urban *molestus* invaded the breeding area of rural *globocoxitus*, hybridization followed. The hybrid population, however, did not endure.

Apparently identical allopatric *molestus* populations may develop subtle but profound differences. In Europe, this form may be divided into several mating groups that do not normally interbreed in nature, as they are geographically distinct (Laven, 1959). However, cytoplasmic sterility factors render the hybrids of certain of these populations non-viable. Various allopatric populations of North American and Philippine origin do not appear to be differentiated in this manner (Rozeboom, 1958).

CONCLUSIONS

C. pipiens is a polytypic species in which there are at least three major adaptations to different environments. The tropical *quinquefasciatus* form is a generalized blood-feeder that is dependent upon the year-round availability of a variety of hosts. The obligate blood-feeding *pipiens* form of the temperate zone appears to be adapted to feeding upon nesting birds. The autogenous *molestus* form is essentially non-blood-feeding and replaces the other biotypes in sheltered, confined locations in cool climates. Additional variants are present in different parts of the world.

The variety of devices that serves to separate these populations poses difficult problems for the taxonomist. The *pipiens* and *quinquefasciatus* forms are isolated geographically and appear to interbreed where their ranges overlap. The same may be said for the *molestus* and *quinquefasciatus* forms. The *pipiens* and *molestus* forms are frequently sympatric

¹ *C. p. quinquefasciatus* Say = *C. p. fatigans* Wiedemann.

and appear to be isolated by mating barriers. These relationships might suggest that three geographical subspecies exist and that they are arranged in a kind of "circle distribution". In such a situation, the *quinquefasciatus* form would serve to link the others.

This is contradicted, however, by the close morphological affinities of the *pifiens* and *molestus* forms. The paradox becomes deeper when one considers that the *molestus* biotype may, itself, represent a complex of non-interfertile populations.

RÉSUMÉ

Le complexe *Culex pifiens* offre des possibilités particulièrement intéressantes dans deux domaines de la recherche biologique fondamentale: les mécanismes entraînant la séparation des populations et les avantages éventuels résultant du prélèvement de repas de sang. L'auteur étudie l'importance de la transmission génétique du caractère obligatoire ou facultatif de cet aspect du comportement et la structure des populations de *C. pifiens*.

Les femelles des biotypes autogènes ou anautogènes pondent un nombre d'œufs caractéristique; si ces dernières sont plus fécondes, en revanche les femelles autogènes peuvent pondre sans être alimentées. Chez les hybrides, le comportement est variable: ils peuvent être autogènes ou anautogènes ou bien encore présenter une autogénie sans maturation des œufs; ils sont, de plus, mal adaptés aux conditions naturelles. Les exigences biologiques de chacun de ces deux groupes commandent leur séparation écologique. Les gîtes larvaires des groupes anautogènes semblent caractérisés par leur grande accessibilité aux femelles gravides. Les potentialités de ponte du biotype anautogène n'atteignent leur maximum qu'après alimentation sur des hôtes aviaires.

Dans la région de Boston (Etats-Unis), les femelles de *C. pifiens* se nourrissent rarement sur l'homme et c'est surtout *C. salinarius*, souvent confondu avec le premier, qui est le plus gênant dans cette région. Bien que souvent décrites comme anthropophiles les populations autogènes piquent rarement l'homme. Pour les populations anautogènes, les oiseaux domestiques sont la principale source

d'alimentation. Une corrélation entre couvain et développement des populations de moustiques a pu être établie. Divers facteurs de milieu conditionnent la croissance relative des deux biotypes.

Dans les régions tropicales, un troisième biotype existe: *C. p. quinquefasciatus*, morphologiquement distinct des deux précédents. Les femelles de *C. p. quinquefasciatus* se nourrissent assez fréquemment sur l'homme et sont d'importants vecteurs de maladies. Alors que dans certaines régions où elles cohabitent les deux sous-espèces se reproduisent isolément, on trouve dans d'autres des individus morphologiquement hybrides. L'étude de la distribution saisonnière de *pifiens* et de *quinquefasciatus* permet d'attribuer au premier une capacité de reproduction plus élevée.

La variété *C. p. pallens* du Japon constitue une quatrième sous-espèce importante du complexe *C. pifiens*. Il s'agit d'un groupe homogène, dont la morphologie est intermédiaire entre celles des formes *pifiens* et *quinquefasciatus*. D'autres variétés ont été signalées dans différentes parties du monde: *globocoxitus* et *australicus*, en Australie, se distinguent par divers caractères d'autres formes largement répandues; en Europe, *molestus* se divise en au moins quatre groupes.

Les rapports entre les groupes de l'espèce *pifiens* suggèrent l'existence de trois sous-espèces ayant une distribution géographique « en cercle ». La diversité des critères d'identification pose au taxonomiste un problème difficile à résoudre.

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