Inversion Polymorphism and Adult Emergence in Anopheles stephensi

Abstract. *The adult carriers of two alternative gene arrangements in* Anopheles stephensi *were found to have distinct daily distributions of emergence, the heterokaryotype being intermediate between the homokaryotypes. Such differences in temporal organization may arise from inversion coadaptation that results in the production of diversified genetic regulatory systems.*

The study of polymorphism caused by inversion of blocks of genes depends on the availability of favorable chromosomes; some families of *Diptera* with polytene chromosomes have been particularly useful in such studies. The most advanced and detailed investigations have been carried out in *Drosophila* mainly by Dobzhansky and his associates (*1, 2*).

Anopheles mosquitoes may also be favorable for the study of inversion polymorphism; polytene chromosomes are found not only in the salivary glands of the larvae but also in ovarian nurse cells of the adult (*3*). The chromosomes in these nurse cells appear to be especially useful for the study of inversion polymorphism in connection with adult behavior. The gene arrangements may be diagnosed in adult females, and comparisons can be made (in the laboratory as well as in the field) between groups of mosquitoes from a polymorphic population sampled according to their responses to certain experimental or ecological situations. In addition to its theoretical interest, the understanding of inversion polymorphism with respect to mosquito biology could have practical implications in epidemiological entomology. We report here on a relation between inversion polymorphism and adult emergence in *Anopheles (Cellia) stephensi* Liston.

The experimental material consisted of four laboratory colonies of *A. stephensi* (*4*) with the following geographic origin: Mamlaha, Iraq; Rahguerd, Iran; Karachi, Pakistan; and Delhi, India. The Indian strain was established more than 20 years ago. The Irani, Iraqi, and Pakistani strains have been maintained in the laboratory since 1960, 1966, and 1968, respectively. Rearing of the mosquitoes was carried out under photoperiod of 12 hours of light and 12 hours of darkness following the technique of Coluzzi *et al.* (*5*) who also describe the details of preparations of nurse cell chromosomes, and give illustrations of the polytene complement of *A. stephensi*.

We studied the paracentric inversion designated as Karachi (KR) that involves the central one-third of the right arm of chromosome 2. The arrangement that was arbitrarily chosen as standard (ST) was the only one present in the Indian strain, the first strain examined. Strains from both Iraq and Iran were found to be polymorphic for the KR and ST gene arrangements (neither of them displacing the other under our laboratory conditions). The heterokaryotype was superior in fitness to the homokaryotype, at least in larval resistance to crowding and in adult longevity. The Pakistani strain was found to be monomorphic KR; recent investigations of other populations of *A. stephensi* indicate that this arrangement is the most widespread, and probably the most primitive, in the species. No other inversion polymorphisms were noted in these four strains, and no evidence of reproductive isolation between them was obtained in the laboratory.

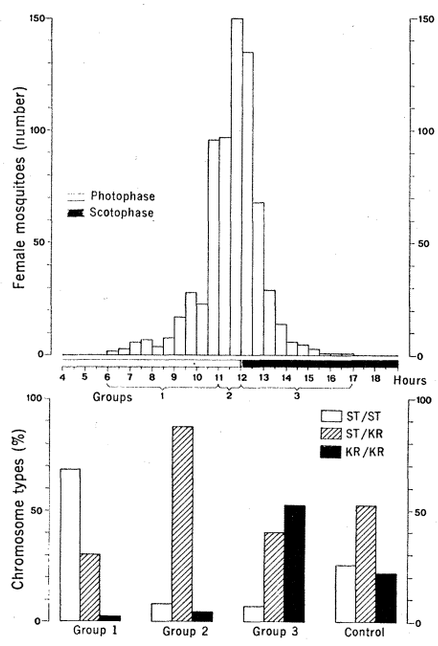

Fig. 1. Distribution of emergence in a sample of female *A. stephensi* divided in three groups according to time of emergence. The chromosome constitution of each group and of the control is illustrated below the emergence curve.

We studied the feeding behavior of the ST/ST, KR/KR, and ST/KR genotypes of the Iraqi strain. Differences were soon noticed in the sugar feeding propensity during the photophase, suggesting the existence of some difference in the circadian cycles of activity. Attention was then shifted to adult emergence, an activity that is more easily recorded and that appears to depend on several rhythmic functions fundamental to the developmental physiology and to the behavior of the insect (*6*). The daily distribution of emergence of adult females of *A. stephensi* peaked around the end of the photophase, but occurred slightly earlier in the males. The persistence of the rhythm was observed in samples maintained in continuous darkness or continuous light from the fourth larval stage on. The shape of the emergence curve differed on consecutive days of emergence and appeared to be influenced by various environmental factors. The 90 percent emergence period, which was restricted to less than 5 hours under nearly optimum rearing conditions, was considerably lengthened when the density of the larval population was increased or when rearing temperatures were lowered.

An experiment was designed to detect possible differences in the pattern of emergence of the three inversion karotypes. About 4,000 first-stage larvae were randomly chosen from more than 10,000 larvae hatching from the eggs laid in one night by a cage population of the Iraqi strain. The larvae were equally distributed in 20 rearing bowls and kept at 27° to 28°C. Almost all of them pupated 7 to 9 days after hatching. Pupation occurred generally in the morning, and the adults emerged in the evening of the next day. The adults emerging on the ninth day from 16 of the rearing bowls were collected every 30 minutes, the pattern of emergence being recorded separately for each sex. The female mosquitoes were divided in three groups according to their time of emergence (Fig. 1). The adult females emerging from the other four rearing bowls were considered the control group. The scotophase was omitted on the ninth day to permit collection of the insects.

The constitution of the chromosomes of the three groups and that of the control were studied in samples of 150 adult females. The actual number of mosquitoes examined was slightly less, as a few chromosome preparations were

Table 1. Chromosome types in three groups of *A. stephensi* adult females with different times of emergence.

Group	Mosquitoes examined (No.)	Frequencies of:					
		ST/ST		ST/KR		KR/KR	
		No.	%	No.	%	No.	%
1	143	98	68.53	42	29.37	3	2.10
2	146	12	8.22	128	87.67	6	4.11
3	139	10	7.19	56	40.29	73	52.52
Control	142	36	25.35	75	52.82	31	21.83

not readable. The results are shown in Fig. 1 and Table 1. Differences in the frequencies of ST/ST, KR/KR, and ST/KR karyotypes were found among the three groups, all of which showed a chromosomal constitution significantly different from that in the control group ($P < .001$). The alternative gene arrangements showed clinal frequency changes from group 1 to 3, indicating a close relation between type of chromosome and the pattern of adult emergence. The mosquitoes emerging first were mostly homozygous ST/ST; the heterozygotes, ST/KR, reached the highest frequency during the middle part of the emergence period; and the homozygotes KR/KR were mainly recorded among those mosquitoes emerging last. In other words, each of the three karyotypes showed a distinct emergence peak, the heterokaryotype being intermediate between the two homokaryotypes.

Because of differences in the period of larval development in the three inversion genotypes, the genotype frequency differed significantly in mosquitoes that emerged on consecutive days. This accounts, in part, for the changes in the shape of the emergence curves. However, the reported relations between the emergence periods of the three inversion karyotypes were recorded on the three principal, consecutive days of emergence in a total of seven replicates of the experiment just described, four of them with the Iraqi strain and three with the Irani strain. Similar results were also obtained in two experiments with the time of pupation. Other experiments indicate the following:

1) The selection of the Iraqi strain for earlier adult emergence resulted in a rapid and highly significant increase in the frequency of ST. Parallel selection for delayed adult emergence resulted in a significant increase of KR.

2) The mean emergence time was different in homozygous ST and KR stains extracted at random (without selection depending on emergence time) from the Iraqi strain. The ST strain showed an early emergence pattern, while the KR strain showed a delayed emergence pattern that also appeared relatively less variable.

3) The differences reported in 2) were not found in the strains from India and Pakistan that were monomorphic for the ST and KR gene arrangements, respectively. The emergence curves of these strains resemble those of the polymorphic strains.

4) The intermediate emergence pattern of the heterokaryotype, typical in the polymorphic strains, was not observed in an F_2 sample obtained from a cross between the KR Pakistani strain and the ST Indian strain.

The evidence indicates that the difference in the emergence time between the carriers of the ST and KR gene arrangements is not determined by the inversion in itself, but is a result of a process of heteroselection, the two homokaryotypes being coadapted in the sense used by Dobzhansky (*7*). The Indian strain is monomorphic for ST and the Pakistan one is monomorphic for KR, whereas the strains from Iraq and Iran are polymorphic. It is likely that, in the polymorphic populations, the chromosomes with the two gene arrangements carry different gene complexes that are coadapted, and that interact favorably in heterozygous carriers. This situation produces a balanced polymorphism and appears to originate the difference in emergence time reported here. If this hypothesis is correct, the process of inversion coadaptation would constitute a genetic device for the production of diversified genetic regulatory systems. Such polygenic variants, determining diverse developmental (and behavioral) pathways, are likely to play a role in colonization of ecologically marginal zones and in the occupation of new ecological niches. This might suggest a causal relation between chromosomal rearrangements and speciation (*2, 8, 9*).

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References and Notes

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Restoration of Antibody-Forming Capacity in Cultures of Nonadherent Spleen Cells by Mercaptoethanol

Abstract. *Populations of mouse spleen cells, without those cells that adhere to glass or plastic, exhibit little or no capacity for formation of antibody to sheep erythrocytes in the Mishell-Dutton culture system. When 5×10^{-5}M mercaptoethanol is added to the culture medium, the antibody-forming capacity of these nonadherent spleen cells is restored to that of unfractionated spleen cells.*

Dissociated spleen cells from normal inbred mice can be separated into two different populations on the basis of their adherence to plastic or glass. Both the adherent and the nonadherent cell populations are required for optimal immune response in vitro to sheep erythrocytes (*1*). The adherent cell population (i) is rich in phagocytes but does not contain the precursors of antibody-forming cells (*2*); (ii) has a function that is radioresistant (*3*); and (iii) can be replaced by appropriate numbers of peritoneal macrophages (*4–6*) or, in some instances, by supernatants of macrophage cultures (*6, 7*). We show here that addition of mercaptoethanol, in low concentrations, to cultures of nonadherent cells restores their capacity to form antibodies in