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Genetica. — *Cage experiments on homospecific and heterospecific matings with females of* Anopheles stephensi *carriers of different inversion karyotypes.* Nota di MARIO COLUZZI (*) e MARIA ANGELA DI DECO (*), presentata (**) dal Socio G. MONTALENTI.

RIASSUNTO. — L'accoppiamento con maschi omospecifici ed eterospecifici è stato studiato sperimentalmente in femmine di *Anopheles stephensi* portatrici di diversi cariotipi di una inversione autosomica paracentrica che comprende la zona centrale del braccio cromosomico 2 R. Le due sequenze alternative *b* e + sono presenti in numerose popolazioni di *A. stephensi* nelle quali determinano polimorfismi bilanciati. La propensione all'accoppiamento delle femmine portatrici dei diversi ordinamenti è risultata simile negli esperimenti con maschi omospecifici mentre evidenti differenze sono state osservate nei saggi con maschi eterospecifici appartenenti alla specie *Anopheles merus* del complesso *gambiae*. La percentuale di femmine inseminate è stata, in tutte le repliche sperimentali, significativamente più alta negli omocariotipi *b/b* che negli omocariotipi +/+ mentre gli eterocariotipi +/*b* hanno mostrato tassi di inseminazione intermedi.

INTRODUCTION

Differences in the mating activity of carriers of different inversion karyotypes has been the subject of various recent contributions dealing with *Drosophila* species (see Spiess [17], and Parsons [13] for reviews of the literature). Suitable experimental material for similar observations may be also found among anopheline mosquitoes. This group of *Diptera* includes many species showing favourable polytene chromosomes both in the larval and in the adult stage while inversion polymorphism is widespread in various populations (see Coluzzi and Kitzmiller [7]). On the other hand the study of intraspecific genetic variability in mating activity in anopheline mosquitoes should have practical implications in the establishment of laboratory colonies and in the development of genetic control procedures (Mattingly [12], Coluzzi [2]).

The observations reported in the present paper deal with the inversion karyotypes in relation to mating activity in female mosquitoes of *Anopheles stephensi* Liston. This species appears to be one of the most promising anopheline material for experimental investigations in view of its fairly good adaptability to laboratory breeding. Available chromosomal data on *A. stephensi* include the description of the polytene complement from larval salivary glands (Rishikesh [15]; Sharma *et al.* [16]) and from ovarian nurse cells (Coluzzi *et al.* [4]). The examination of laboratory colonies from different areas of the Oriental region showed the existence of polymorphisms

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involving six different paracentric inversions three of which on chromosome arm 2 R, one on 2 L and two overlapping on 3 L (Coluzzi *et al.* [6]). The polymorphism by inversion 2 R*b*, apparently the most widespread, is the one studied in relation to mating activity. Previous observations on the *b*/+ inversion polymorphism indicate that the alternative arrangements affect the time of pupation and adult emergence (Coluzzi [3]), biometrical characters such as egg size (Coluzzi *et al.* [5], [6]) and cyrcadian flight activity (Jones [11]).

Both homospecific and heterospecific crosses were carried out in the attempt of detecting eventual differences in mating activity between females of *A. stephensi* carriers of different 2 R*b* inversion karyotypes. After preliminary trials with males of *A. superpictus*, *A. rufipes*, *A. merus*, *A. melas* and *A. gambiae species B*, the material of *A. merus* (one of the sibling species of the *gambiae* complex) was considered the most promising and utilized in the heterospecific crosses.

Materials and methods

The material of *A. stephensi* was derived from the strain known as Iraq-1966 established from adult females collected in the Fao area on the Iraqui coast of the Persian Gulf. Informations on this strain (including insecticide resistance, crossing experiments and chromosomal studies) were reported by De Zulueta *et al.* [10], Coluzzi *et al.* [4], [5], [6] and Coluzzi [3]. The 2 R*b* inversion which lies in the central part of the chromosomal arm 2 R including 0.31 of its total length, was the only one detected in the Iraq-1966 strain. The alternative arrangements *b* and + (previously indicated as *Standard* and *Karachi* respectively) were found to have similar frequencies forming a typical balanced polymorphism (Coluzzi [3]; Coluzzi *et al.* [6]).

The following homokaryotypic strains were extracted from the laboratory population by breeding separately the progenies of single females.

Strain 14+, obtained by intercrossing 14 families homozygous +/+.
Strain 18b, obtained by intercrossing 18 families homozygous *b*/*b*.
Strain 9+, obtained by intercrossing 9 families homozygous +/+.
Strain 11b, obtained by intercrossing 11 families homozygous *b*/*b*.

These strains were maintained as the parent laboratory population with each generation involving not less than 400 specimens crossing at random.

The observations on mating activity were carried out with the homokaryotypic strains *14+* and *18b* while strains *9+* and *11b* were only used in one experimental replica to ascertain the concordance of the results. The heterokaryotypic mosquitoes were obtained by mass crosses of the homokaryotypic strains. Both reciprocal crosses were effected and their F_1 progenies were used separately.

The heterospecific males of *Anopheles merus* were from a laboratory strain established from adult females collected near the Pangani river estuary in Tanzania. Genetical data on this strain are reported by Davidson [9] and Coluzzi and Sabatini [8]. *A. stephensi* and *A. merus* are allopatric species belonging to closely related groups (*Neocellia* and *Pyretophorus*) of subgenus *Cellia*. Species of such groups are sympatric both in the Ethiopian and in the Oriental region showing obvious reproductive isolation which is expected would exist in nature also between *A. stephensi* and *A. merus*. The mating barrier is broken down in the laboratory and females of *A. stephensi* are normally inseminated by males of *A. merus*. The total inseminations (e.g. spermatheca full of spermatozoa) recorded in almost all cases, should indicate the absence of structural difficulties in mating between these species. The sterility barrier was found to be complete, the hybrid zygote being not produced or dying at an early stage, before hatching.

Breeding and experimental observations were carried out in a constant temperature/humidity room (temperature: 27 ± 1 °C; relative humidity: $70 \pm 5\%$) with fixed photoperiod of 12 hours of light. The larvae were reared in plastic bowls (cm $35 \times 25 \times 8$) filled with well water to a dept of 3 cm. The larval food given daily consisted of a mixture of equal parts of finely minced dog pellets ("Gaines meal") and bran. The larval density was adjusted to provide about 3 sq cm of water surface per larva. Such a relatively low density reduces competition and allows uniform and rapid growth. The adults were kept in cubic cages (cm $25 \times 25 \times 25$) with 5 % of sucrose solution constantly available and the females were fed on guinea pigs.

The materials of *A. stephensi* and *A. merus* were found to mate successfully in laboratory cages showing male swarms and coupling on the wing, quite consistently with the natural behavior of *A. stephensi* as described by Quraishi [14]. No attemps were made to evaluate differences in mating activity by direct observations of the rapid behavioral sequences on the wing which leads to copulation. The experimental approach adopted consisted in the indirect evaluation of mating activity by examining the frequency of female insemination after a fixed period of exposure to the males. The observations were carried out using active males 2-6 days old and virgine females emerged in the night before the beginning of the test. In each experiment the ratio was 1 : 1 and the total number of mosquitoes 100 maintained in cubic cages of cm $18 \times 18 \times 18$. The cages were placed inside expanded-polystirene boxes to reduce the influence of uncontrolled environmental factors in the insectary. Sucrose solution was the only food made available to the mosquitoes during the experiment. Female insemination was evidenced by dissection of spermatheca in saline and by examination in phase-contrast. When tested in pairs, in a single cage, the females carriers of different karyotypes were marked by fluorescent dusts ("Signal green A-18", "Horizon blue A–19" and "Arc-yellow A-17" were used alternatively for each karyotype). Marking was obtained without anaesthesia by allowing newly emerged females to fly into a paper cup with dusted walls.

Experimental observations

The results of parallel homospecific and heterospecific crosses with females of *A. stephensi* carriers of different 2R*b* inversion karyotypes are summarized in Table I and II. Table I refers to a first set of experiments in which each female karyotype was tested separately. Table II refers to a second set of experiments in which the female karyotypes were tested in pairs by placing equal number of them in a single cage after being marked with fluorescent dusts. The period of exposure of the females to the males is expressed in number of nights taking into account that in these species mating is a crepuscolar and nocturnal activity and that in the laboratory it follows a cyrcadian rhythm with the peak at the beginning of the scotophase.

Table I

Female insemination in Anopheles stephensi *carriers of different* 2 R *gene arrangements in cage experiments with homospecific or heterospecific* (Anopheles merus) *males. Each female karyotype is tested in separate cages.*

Duration of the experiment (nights)	Males	Female karyotype	Females dissected	Inseminated		χ^2	P
				Number	Percent		
1	*stephensi* (+/+ and *b/b*)	+/+	99	49	49.49	0.13	>0.70
		b/b	98	45	45.92		
	merus	+/+	85	25	29.41	5.46	<0.02
		b/b	90	43	47.77		
2	*stephensi* (+/+ and *b/b*)	+/+	98	82	83.67	0.17	>0.60
		b/b	97	78	80.41		
	merus	+/+	89	38	42.70	8.16	<0.01
		b/b	89	58	65.17		
3	*stephensi* (+/+ and *b/b*)	+/+	89	72	80.89	2.57	>0.10
		b/b	93	84	90.32		
	merus	+/+	190	98	51.58	19.37	<0.001
		b/b	179	133	74.30		

TABLE II

Female insemination in Anopheles stephensi *carriers of different* 2R *gene arrangements in cage experiments with homospecific or heterospecific* (Anopheles merus) *males. The female karyotypes are tested in pairs by placing equal numbers of them in a single cage.*

Duration of the experiment (nights)	Males	Female karyotype	Females dissected	Inseminated		χ^2	P
				Number	Percent		
2	*stephensi* +/+	+/+	99	77	77.78	0.01	>0.90
		b/b	97	77	79.38		
	stephensi *b/b*	+/+	99	71	71.72	1.26	>0.20
		b/b	98	78	79.59		
	merus	+/+	116	42	36.21	15.19	<0.001
		b/b	113	71	62.83		
3	*merus*	+/+	155	49	31.61	36.26	<0.001
		b/b	155	103	66.45		
	merus	+/+	114	42	36.84	11.04	<0.001
		+/*b*	112	67	59.82		
	merus	*b/b*	108	74	68.52	16.16	<0.001
		+/*b*	111	45	40.54		

In the intraspecific cross with *A. stephensi* the rate of female insemination was found to reach about 80 % after the second night. The intraspecific cross with *A. merus* (not reported in the Tables) showed very similar results. These figures are in agreement with observations on samples of both *A. stephensi* and *A. merus* taken from the respective breeding cages showing insemination rates frequently above 90 %. Considering the relatively low insemination rates recorded in the one-night tests it should be remembered that females were emerging during the previous night and that part of them may have been not yet sexually receptive. The one-night test with *b/b* homokaryotypic females provided the only example of similar insemination rates by homospecific ad heterospecific males. Otherwise the rates of insemination were always comparatively lower in the crosses with *A. merus* indicating the effect of a mating barrier which is only partially broken down in the laboratory cages.

The females of *A. stephensi*, carriers of different inversion karyotypes, showed fairly similar insemination rates when crossed to homospecific males while highly significant differences were recorded in the interspecific crosses. The frequency of insemination by males of *A. merus* was found in all experimental replicas to be significantly higher in the *b/b* homokaryotypes than in the +/+ homokaryotypes while intermediate figures were obtained with the heterokaryotypic females. The two sets of results reported in Table I and II are remarkably similar in their significance. Comparing the figures for the heterospecific crosses, a greater degree of divergence is noted in the insemination rates of the homokaryotypes when these are tested together in the same cage.

Discussion

The main finding reported in the present paper is that females of *A. stephensi* carriers of alternative gene arrangements on chromosome 2 R show different rates of insemination when exposed to males of *A. merus*. This indicates the existence of relationships between inversion karyotypes and female characters which affect the success of the heterospecific mating. The same characters could influence the intraspecific crosses in polymorphic populations of *A. stephensi* even if this is not revealed by our limited experimental observations.

Assuming female receptivity to be the deciding factor in mating success, the observed differences in the rates of insemination of *A. stephensi* females should reflect different levels of rejection towards the *merus* males. This would involve a recognition of the heterospecific males by their epigamic behavior, presumably " aberrant ", or by the lack of a specific behaviorally active substance (pheromone). However alternative interpretations can be proposed without the need to invoke differences in female sexual behavior. The different rates of insemination could depend on differences of female size and/or vigor or they could be caused by patterns of behavior not directly related to mating but resulting in differences in the activity of the females or in their distribution in the cage environment. Even the hypothesis of a discriminating capacity of *merus* males towards the genetically differing females cannot be discarded at this stage. Direct observations on mating behavior clearly constitute an essential step in order to find support for one or the other of these interpretations.

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REFERENCES

[1] A. N. CLEMENTS (1963) – *The physiology of mosquitoes,* Pergamon Press, London.

[2] M. COLUZZI (1971) – *Problèmes théoriques et pratiques liés à l'élevage et à la production de masse de Culicides,* « Annales de Parasitologie (Paris) », *46*, 91.

[3] M. COLUZZI (1972) – *Inversion polymorphism and adult emergence in* Anopheles stephensi, « Science », *176*, 59.

[4] M. COLUZZI, G. CANCRINI and M. DI DECO (1970) – *The polytene chromosomes of* Anopheles superpictus *and relationships with* Anopheles stephensi, « Parassitologia », *12*, 101.

[5] M. COLUZZI, G. CANCRINI and M. DI DECO (1972) – *Polimorfismo cromosomico e lunghezza dell'uovo in* Anopheles stephensi, « Parassitologia », *14*, 261.

[6] M. COLUZZI, M. DI DECO and G. CANCRINI (1973) – *Chromosomal inversions in* Anopheles stephensi, « Parassitologia », *15*, 129.

[7] M. COLUZZI and J. B. KITZMILLER (1975) – *Genetics of anopheline mosquitoes,* « Handbook of genetics », Plenum Pub. Co., New York.

[8] M. COLUZZI and A. SABATINI (1969) – *Cytogenetic observations on the salt water species,* Anopheles merus *and* Anopheles melas, *of the* gambiae *complex,* « Parassitologia », *11*, 178.

[9] G. DAVIDSON (1964) – *The five mating types in* Anopheles gambiae *complex,* « Riv. Malariol. », *43*, 167.

[10] J. DE ZULUETA, T. L. CHANG, J. R. CULLEN and G. DAVIDSON (1968) – *Recent observations on insecticide resistance in* Anopheles stephensi *in Iraq,* « Mosquitoes News », *28*, 499.

[11] M. S. R. JONES (1974) – *Inversion polymorphism and cyrcadian flight activity in the mosquito* Anopheles stephensi List. (Diptera, Culicidae), « Bull. ent. Res. », *64*, 305.

[12] P. F. MATTINGLY (1967) – *Genetics of behavior,* « Genetics of insect vector of disease », Elsevier Amsterdam, 553.

[13] P. A. PARSONS (1973) – *Behavioral and ecological genetics,* Clarendon Press, Oxford.

[14] M. S. QURAISHI (1965) – *Swarming, mating and density in nature of* Anopheles stephensi mysorensis, « J. econ. Ent. », *58*, 821.

[15] N. RISHIKESH (1959) – *Morphology and development of the salivary glands and their chromosomes in the lavae of* Anopheles stephensi sensu strictu, « Bull. Wld Hlth Org. », *30*, 47.

[16] G. P. SHARMA, R. PARSHAD, S. L. NARANG and J. B. KITZMILLER (1969) – *The salivary chromosomes of* Anopheles stephensi stephensi, « J. med. Ent. », *6*, 68.

[17] E. B. SPIESS (1970) – *Mating propensity and its genetic basis in* Drosophila, « Essays in evolution and genetics in honor of Theodosius Dobzhansky », North-Holland Publ. Co., Amsterdam, 315.