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SHORT COMMUNICATIONS

Variability of Centromeric Chromatin in Chromosome 2 of Ovarian Nurse Cells in Inbred Mosquito *Anopheles Atroparvus* V. Tiel

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Abstract—We investigated the variability of pericentromeric chromatin of chromosome 2 in ovarian nurse cells (trophocytes) in two laboratory lines of malaria mosquito *Anopheles atroparvus* V. Tiel and in their hybrids. One line had been raised by means of sib inbreeding, the other kept at constantly high population density. The inbreeding was shown to result in an increased percentage of chromosomes bearing an achromatinic zone in the centromeric region, which resulted in chromosome breakage. Toxicological tests demonstrated an increase in the sensitivity of the progeny of females with abnormal morphotypes of chromosome 2 to the entomopathogenic bacterium *Bacillus thuringiensis israelensis*. The appearance of the achromatinic zone is attributed to local chromatin underreplication accompanying chromosome polytenization. Possible reasons for this phenomenon and its implication for adaptation are discussed.

Heterochromatin modifications are commonly known to correlate with adaptation to diverse living conditions [1–3]. Studies on induced heterochromatin variability, caused by adverse factors, are of particular interest. For example, it was shown that heterochromatin could produce various morphological changes in response to inbreeding [4, 5]. In the present paper, we report the changes in the morphology of the pericentromeric chromatin of polytene chromosomes caused by inbreeding in a laboratory line of malaria mosquito *Anopheles atroparvus* V. Tiel and the correlations between these changes and adaptive features of the individual. The resistance of the larvae to the entomopathogenic bacterium *Bacillus thuringiensis subsp. israelensis* (*Bti*) was used as an adaptation index.

Two laboratory lines of *Anopheles atroparvus* were used. One of them (I) had been raised by sib inbreeding, the other (N) has been raised under constantly high population density and was kindly provided by workers of Martsinovskii Institute. Squash preparations were done following the conventional lactate–acetoorcein procedure [6]. Clones of the crystal-forming bacterium *Bti*, serovar H14, were used for testing *A. atroparvus* larvae for bacterial resistance. The bacterial culture was grown as in [7]. The *Bti* suspension of the biotests was prepared by dilution of 100 μl of the starting broth (73.375 mg/ml) in 2.3 ml of water. *A. atroparvus* larvae were taken from first synchronous ovipositions (from each individual female) and infected with *Bti* at the fourth instar. The tests were done in plastic trays of sizes 21 × 10 cm. In the first case, 50 larvae in 200 ml of dechlorinated water were placed per replication and infected by 40 μl of the experimental solution. In the other case, the larvae were placed 25 individuals per replication in 200 ml of dechlorinated water. Surviving larvae were grown to the adult stage, and chromosomes of ovarian nurse cells (trophocytes) were examined.

Pericentromeric heterochromatin (PH) is the most variable part of the genome in malaria mosquitoes *Anopheles maculipennis*. Interspecific variations [8] and intraspecific polymorphism [9] have been discovered in the morphology of the PH of polytene chromosomes of ovarian trophocytes in this complex. Interspecific variability of PH in *Anopheles atroparvus* was found only in chromosome 2. PH of this chromosome is known to have a diffuse, nonhelical structure [8]. In situ hybridization with labeled highly repetitive DNA showed an intense labeling of the entire non-helical chromatin zone (Figs 1b–1e).

Variability of centromeric chromatin, found in *A. atroparvus*, manifested as an additional achromatinic zone between the arms of this chromosome in the range from 14c to 15d (Figs 1d, 1e). The sizes of the achromatinic zone varied from 1–3 μm up to complete breakage of the arms, characteristic of the species, in which chromosome 2 is tightly attached to the nuclear membrane [10, 11]. It is worth noting that the breakage was not caused by the mechanical effect of the preparation squashing. It can be clearly seen in Fig. 1b that arm divergence in the centromeric zone of this region stretches, but is not torn. In the other case, the area of the breakage is clearly seen, even in the absence of chromosome tension in this area (Fig. 1d).

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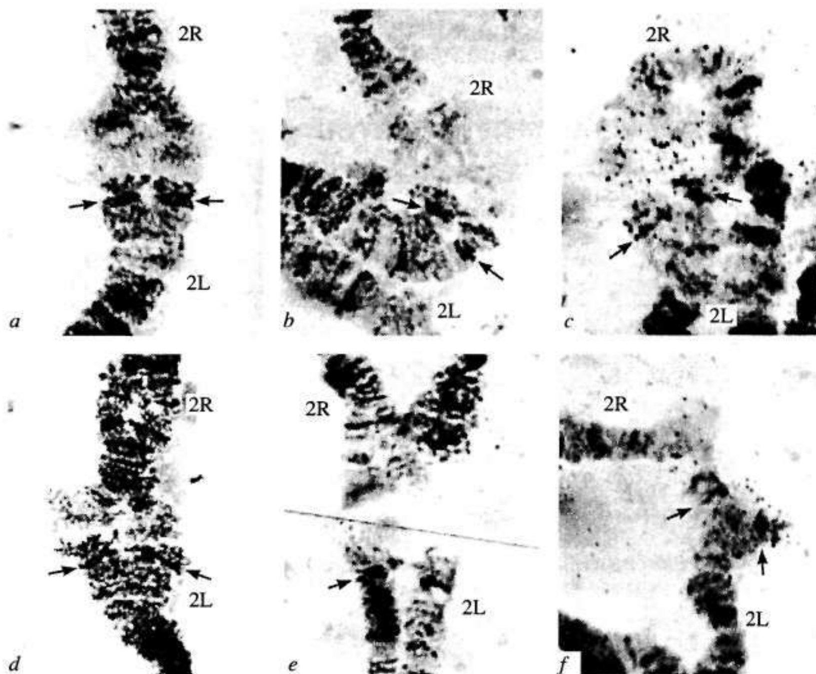

Fig. 1. Morphological traits of PH of chromosome 2 in trophocyte nuclei of *A. atroparvus*: (a–c) absence of arm divergence (normal morphotype); (d–f) arm divergence in the stretch 14c–15d (abnormal morphotype); (c, f) localization of silver particles in the pericentromeric heterochromatin after in situ hybridization in highly repetitive DNA sequences.

Three types of PH morphology of chromosome 2 in *A. atroparvus* were defined to describe the phenomenon. The original type, that is, complete absence of arm divergence, was recognized as the normal morphotype. The two abnormal morphotypes included: the initial stage of arm divergence (Fig. 1d), where the distance between the arms is 1 to 3 μm, and the complete separation of the arms (Figs 1e, 1f). To reveal the causes for this, we compared morphotypes for chromosome 2 in two *A. atroparvus* laboratory lines and crosses between them. Significant differences between the lines were found (Tables 1, 2).

Line I, raised under the conditions of sib inbreeding, was characterized by deviations from the norm, apparently prevailing in the noninbred line N. It is of interest that crosses between the lines demonstrated a dramatic shift towards the domination of the norm. This comparison revealed also a maternal effect in the inheritance of chromosome 2 morphology. Crosses with the female from the noninbred line N produced more normal variants of CH morphology than crosses of Line I females with noninbred males. The relative increase in the percentage of the norm was calculated to be 40.9% in the first case and 36.8% in the second.

Table 1. Quantitative evaluation of the variation in chromosome 2 morphology in trophocytes of *A. atroparvus*

A. atroparvus females *A. atroparvus* line	Number of nuclei	Morphology of chromosome 2 (%)		
		norm	the initial stage of divergence	complete divergence
Inbred (I)	90	47.8 ± 5.29	40.0 ± 5.19	12.2 ± 3.74
Normal (N)	162	60.6 ± 3.84	36.4 ± 3.78	3.0 ± 1.34
N × I	122	85.2 ± 3.20	14.8 ± 3.20	0
I × N	107	65.4 ± 4.60	33.6 ± 4.57	1.0 ± 0.96

Table 2. Comparison between the inbred and normal lines of *A. atroparvus* and crosses between them ($P < 0.05$)

Line	Chi-square χ^2 (*d.f.* = 2)			
	inbred (I)	normal (N)	N × I	I × N
Inbred (I)	–	9.51	38.37	13.43
Normal (N)	0.01	–	21.82	1.73
N × I	0.001	0.001	–	12.71
I × N	0.01	0.9	0.01	–

Table 3. Cytological features of *A. atroparvus* females with "normal" and "abnormal" morphotypes

Phenotype of the female	Variation in $(x - y)/(x + y)$		Number of females
	min.	max.	
Normal	+26	+67	4
Abnormal	–50	–33	5

Table 4. *Bti* sensitivity in the progeny of *A. atroparvus* females with normal and abnormal morphotypes

Phenotype of the female	Variant of the experiment	Number of larvae	Mortality, %	LD_{50}, μl per larva
Normal	Experiment	189	13.2 ± 2.5	2.52
	Control	82	5.9 ± 3.3	
Abnormal	Experiment	234	36.8 ± 3.2	2.02
	Control	99	19.0 ± 3.9	

It was of interest was to find out if the abnormal morphotype was related to other manifestations of inbred depression. We tested the inbred *A. atroparvus* line for resistance to the entomopathogenic bacterium *Bti*. Since the investigated character was not strictly discrete and found to be variable over the preparation, we determined the phenotype of the female using the approximation $(x - y)/(x + y)$. If the expression was positive, the female was determined by convention as normal, if negative, abnormal (Table 3).

The progeny of abnormal females was less viable over all the investigated indices. The death rate in the progeny of abnormal females was higher both in the experimental ($P < 0.001$) and control populations. The death rate did not differ between the experimental and control populations in the progeny of normal females, but showed a significant difference in the progeny of abnormal ones ($P < 0.05$) (Table 4).

Thus, the presence of a modified PH morphotype for chromosome 2 in trophocytes of the female parent correlated with decreased viability of the progeny and worse physiological adaptability in general. In other words, the laboratory *A. atroparvus* lines demonstrated correlations between PH properties and adaptive indices of the individuals.

Similar changes were found to be caused by inbreeding in *Calliphora eritrocephala*, where polytene chromosomes with achromatinic zones were seen in centromeric regions of some trophocytes in females [12]. Heterochromatin changes were observed on inbreeding in maize [4, 5]. It was shown that the pachytene chromosomes of this plant contained so-called heterochromatin node regions (HNR), whose number and location was strictly constant within particular lines. Upon inbreeding, various changes occurred in these regions in some meiocytes: diffuse and pufflike patterns of previously compact node regions, unequal exchanges between homologous HKRs, ectopic coupling, and paracentric inversions. The authors concluded that all these changes were caused by a delay of DNA replication taking place during inbreeding, and the inbred depression is the result of these events.

A specific underreplication of heterochromatin DNA may also take place in *A. atroparvus* and manifest as an achromatiic zone. It is likely that when the population experiences inbreeding and the corresponding genome homozygotization, heterochromatin changes provide additional variability. Thus, heterochromatin appears to be a versatile system, able to respond to external impacts.

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