Possible Genetic Consequences of Meiosis in Pocket Gopher (*Thomomys bottae*) Populations

Variation in mitotic chromosomal pattern is perhaps more extensive in the single species of pocket gopher, *Thomomys bottae* (Eydoux and Gervais), than in any other species of mammal as yet examined. The variation is limited to changes in chromosome morphology and is not complicated by concommitant variation in diploid number. Both interpopulation polytypic conditions and intrapopulation polymorphism have been recorded[1-3]. Populations are known to possess from 0 to 32 acrocentric autosomes, and adjacent populations have been described which differ by up to 9 fixed chromosomal rearrangements[4].

The major mechanism of chromosomal transformation considered responsible for these observed levels of diversity is that of pericentric inversions. Theoretically, individuals heterozygous for one or more such inversion should be at least partially sterile due to the production of from 50 to 100% unbalanced gametes resulting from crossing-over within the inverted segments. Evidence to date, however, suggests no reduction in fertility in heterozygous individuals. Numerous chromosomal heterozygotes collected from the zones of contact between adjacent but karyotypically distinct populations have been examined[2,4]. Moreover, heterozygosity for from 3 to 5 separate inversions is apparently maintained under balanced conditions in some instances[3,4]. The present report, therefore, considers some aspects of meiosis among karyotypically diverse populations of this species, aspects which are intimately related to the levels of chromosomal variation recorded and to the maintenance of reproductive success even in chromosomal heterozygotes.

Meiotic preparations were obtained from testicular material only, following the procedure outlined by FORD[5]. 78 male *Thomomys bottae* representative of 6 different subspecies and 8 separate populations were examined. These populations differed by the presence of from 0 to 18 acrocentric autosomes or, consequently, from 0 to at least 9 fixed pericentric inversions. In all cases the diploid number was 76. Metaphase somatic karyotypes from the two extremes are given in Figure 1: A) *T. b. modicus* with a completely biarmed autosomal complement and, B) *T. b. alienus* with 9 pairs of acrocentric and only 28 pairs of biarmed autosomes.

1123 cells in first meiotic division were examined, for an average of 14 cells per individual. For chiasma frequency determinations only diakinesis and metaphase I figures

[1] J. L. PATTON and R. E. DINGMAN, J. Mammalogy *49*, 1 (1968).
[2] J. L. PATTON and R. E. DINGMAN, Cytogenetics *9*, 139 (1970).
[3] J. L. PATTON, Chromosoma *31*, 41 (1970).
[4] J. L. PATTON, in preparation.
[5] C. E. FORD and E. P. EVANS, in *Comparative Mammalian Cytogenetics* (Ed. K. BENIRSCHKE; Springer-Verlag, New York 1969), p. 461.

Fig. 1. Somatic karyotypes of 2 specimens of *Thomomys bottae* representing the range of variation in chromosome morphology considered in this report: A) female *T. b. modicus* (UA 14992) from Yerba Buena Ranch, Patagonia Mts., Santa Cruz Co., Arizona, with an all biarmed autosomal complement; and B) female *T. b. alienus* (UA 14989) from near St. David, Cochise Co., Arizona, with 9 pairs of acrocentric autosomes.

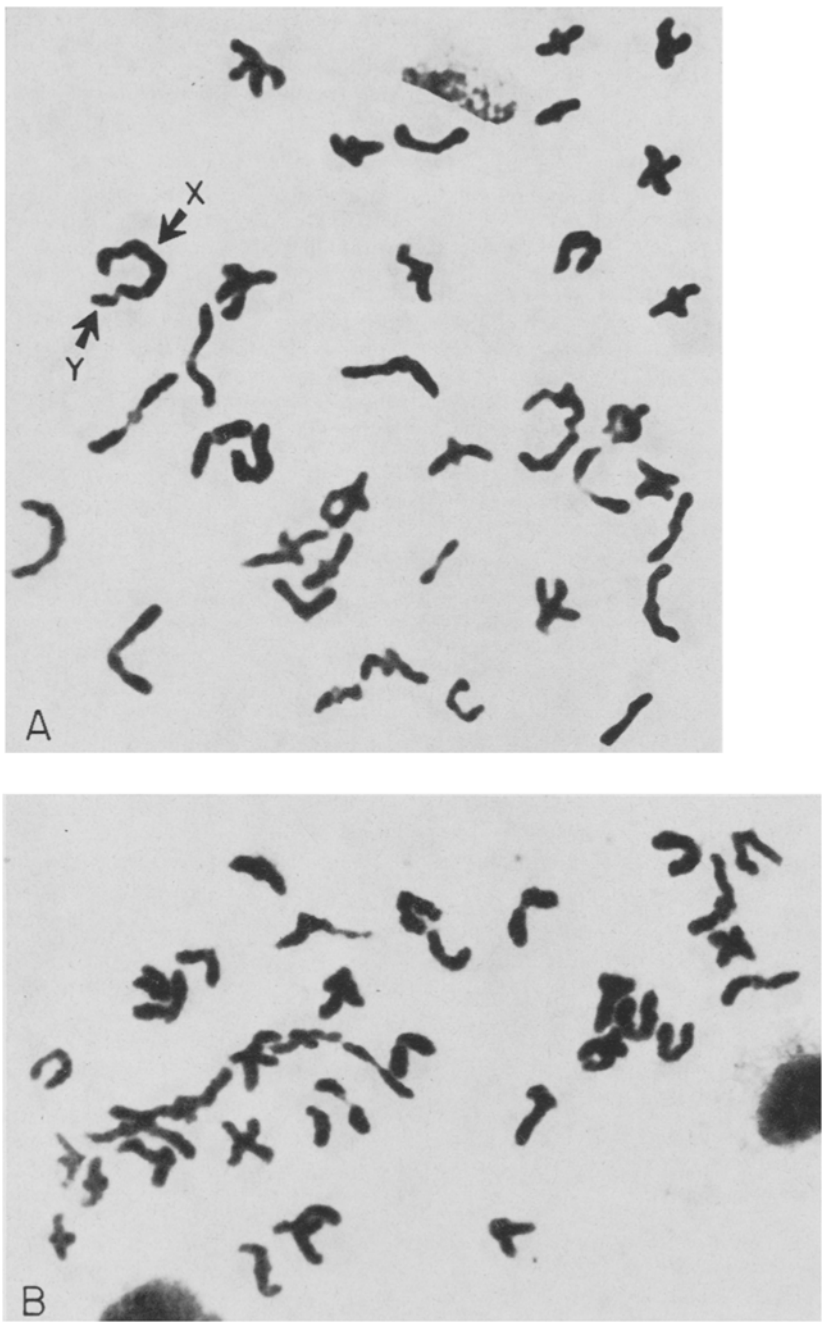

Fig. 2. Representative spermatocytes at diakinesis of 2 chromosomal variants of *Thomomys bottae:* A) specimen of *T. b. modicus* (MVZ 137257) with the same somatic karyotype as pictured in Figure 1A; and B) specimen of *T. b. alienus* (MVZ 139042) with the same somatic karyotype as pictured in Figure 1B.

were scored. This was due to the technical difficulties in separation and resolution of individual bivalents in earlier stages. All specimens examined are characterized by 38 bivalents ($n = 38$), as was expected. Moreover, the general characteristics of bivalent association at diakinesis or metaphase I was the same for all individuals regardless of the differences in individual chromosome morphologies of the somatic karyotypes. These features of similarity are shown in Figure 2 where first metaphase cells from individuals with zero acrocentric autosomes (Figure 2A) and with 18 acrocentric autosomes (Figure 2B) are compared. Note that the majority of bivalents are associated by only a single chiasma which is nearly always located in a terminal or near terminal position. The mean chiasma frequency per bivalent is 1.03 when all individuals examined are pooled (range, 1.00 to 1.05). Chiasma formation appears, therefore, to be limited in number and highly localized in position in a majority of chromosomes. The pattern of chiasma formation is totally independent of the morphology of the individual chromosomes (i.e., whether they are biarmed or uniarmed).

Localization of chiasmata may represent a highly significant feature with regard to the evolution of inter- and intrapopulation chromosomal variability within the species. For one, the evidence suggests that most heterozygous pericentric inversions would produce little or no deleterious load on a population due to the exclusion of crossing-over from within the inverted segments. Consequently, populations can afford large numbers of heterozygous inversions with relatively little gametic penalty. Moreover, and possibly of greater significance, is the influence which the reduction in chiasmata number and

localization may have on the genetic structure of populations and individuals. Such a mechanism would allow for the capture of co-adapted gene sequences which would not be continually disrupted by recombination within them. This may be of immense selective value in adapting local demes to the highly localized ecological conditions which they face, a characteristic of fossorial mammals in general and of pocket gophers in particular[4,6]. Secondly, it would allow for gene exchange between adjacent but karyotypically distinct populations as a means of inputting genetic variability into a population through selective introgression. Finally, it may allow for the maintanence of genic heterozygosity, even in small populations faced with strong inbreeding, a decidedly advantageous feature (see CARSON[7] for review). It has been shown elsewhere that, despite expected reduction in genic heterozygosity resulting from characteristics of the fossorial life mode, populations of *T. bottae* are as genically variable as other less restricted rodent species[8].

Resumen. El analisis de la primera división meiótica des roeder fosorial, *Thomomys bottae*, muestra una uniformidad notable en la colocación de quiasmas en los bivalentes, a pesar de las diferencias existentes en los cariotipos somáticos. La frecuencia promedio de quiasmas por bivalente es muy baja (1.03), y la mayoria de los bivalentes demuestran que sus quiasmas se localizan en o cerca de un estremo del par.

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[6] T. E. KENNERLY JR., Texas J. Sci. *6*, 297 (1954). – C. S. THAELER JR., Univ. Calif. Publ. Zool. *86*, 1 (1968).

[7] H. L. CARSON, in *Evolutionary Biology* (Eds. T. DOBZHANSKY, M. K. HECHT and W. C. STEERE, Appleton-Century-Crofts, New York 1967), vol. 1, p. 143.

[8] J. L. PATTON, R. K. SELANDER and M. H. SMITH, in preparation.

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A Simple Technique for Obtaining Carbon Replicas from Small Organisms

Carbon replicas are widely used for surface studies. Depending on the properties of the subject to be replicated, different methods can be applied, which can be mainly divided into single- and two-stage methods[1].

The advantage of the single-stage method is the relatively small risk of artifacts. The two-stage method, on the other hand, is more laborious but has the advantage of leaving the surface structure undisturbed, thus enabling repeated study of the same surface.

In the single-stage method different techniques were employed to separate the carbon layer from the specimen. Unfortunately these techniques failed in the case of small organisms like aphids. The two-stage method also proved to be unsuccessful since the waxy surface structure is embedded and soluble in the replicating plastic, whereas a water-soluble plastic cannot be employed on account of the small dimensions.

To overcome the difficulties caused mainly by the small dimensions of the organism, a substratum which fixes the material was used.

Method. The procedure of replication can be divided into 6 steps: 1. A mixture of the powder and fluid of Technovit®/4071-d (KULZER & Co., 638 Bad Homburg, P.O. Box 261, West Germany) is prepared according to the directions for use. 2. A glass slide is covered with the mixture. 3. During the few minutes in which the plastic hardens, the organisms can be placed on it with a painting brush. Care must be taken to avoid contact of the surface to be replicated with the resin. 4. The dried preparation is now ready for carbon deposition (100 Å) in the vacuum plant. To prevent excessive shrinking the recommandations of Bradley, in Kay[1], concerning leaf replicas must be kept in mind: a reasonably short pumping time and a vacuum not much higher than 10^{-3} mm Hg. 5. To dry-strip the carbon film, a 4% solution of collodion in an anhydrous mixture containing equal parts of ether and ethyl alcohol is dripped on the assembly. The collodion must be dried at room temperature under conditions of low relative humidity. 6. The collodion-carbon film is then cut into small pieces, the collodion is subsequently dissolved in ether/alcohol, the replicas are mounted on grids (preferably 200 mesh/in, VECO, Holland) and shadowed if required.

The materials replicated in this study were abdomen and wing of the mealy plum aphid, *Hyalopterus pruni* (Geoff.) and leaves of the common reed, *Phragmites communis* Trin.

Result and discussion. The dry-stripping (stage 5) results in a collodion film with carbon only from the surfaces to be replicated and transparent on the places above the substratum, since the adhesive forces between Technovit and carbon are stronger than between carbon and collodion.

The carbon can be dry-stripped from an organism with a waxy surface layer by means of collodion, but it must be admitted that the wax and the carbon are not separated. In this procedure the wax is torn from the surface of the organism resulting in a collodion/carbon film encrusted with wax, a material which indeed is partly dissolved in the ether/alcohol mixture, but nevertheless contributes to an electron image with enhanced contrast. The adhesive properties of the substratum and the collodion 1. preclude ambiguity caused by replica picture of the Technovit, 2. eliminate an extra step of shadow casting which would be used only for the purposal of improving the picture, 3. but they make it impossible to obtain a replica from an organism of which the waxy layer is completely corroded, e.g., by mechanical or microbial action.

Another property of the collodion in which we were highly interested was the possibility of shrinkage. Therefore, we replicated leaf surfaces both in the usual way[2] and by employing the method described. As can be gathered from the photographs (Figures 1, 2), both techniques resulted in similar images. We thus concluded that the technique developed is satisfactory for the replication of small

[1] D. H. KAY, *Techniques for Electron Microscopy*, 2nd edn. (Blackwell Scientific Publications, Oxford 1965).

[2] B. E. JUNIPER and D. E. BRADLEY, J. Ultrastruct. Res. *1*, 16 (1958).