Homozygous Reciprocal Translocation as a Mode of Speciation in *Microgale* Thomas 1883 (Tenrecidae-Insectivora)

During the course of our cytotaxonomic studies on the insectivore family Tenrecidae Gray 1821 we recently investigated 2 species of *Microgale* O. Thomas 1883: *M. dobsoni* O. Thomas 1884 and *M. talazaci* F. Major 1896. These 2 species, as all the other species of tenrecs, are found only on the island of Madagascar. THOMAS[1] erected a new genus *Nesogale* to accomodate these 2 species but following SIMPSON[2] we will refer them to the genus *Microgale* for the present paper. One male specimen of *M. dobsoni* reported here was collected in March, 1966[3] near Manandroy (21°9′S, 47° 15′ E) and 5 females of *M. dobsoni* were collected in April, 1967 near Manohilahy (19° 17′S, 48° 01′E). Three females and 7 males of *M. talazaci* were collected from February to April, 1967 near Perinet (18° 54′S, 48° 24′E). The animals were taken in Sherman aluminum live-traps.

The chromosome studies were performed on whole blood, spleen, and skin fibroblast cultures by standard techniques[4,5]. Tissue samples were mailed by air in cool (35–40°F) containers from Perinet, Madagascar to Baltimore and then processed. Meiotic studies were conducted on testicular material obtained from animals in Baltimore by following a modification of the technique of EVANS et al.[6]. Cells from different tissues of all the animals gave a count of $2n = 30$ chromosomes (Table). Karyotypes prepared from different tissues of animals in each species show no variation (Figures 1 and 2). At meiosis the chromosomes seem to associate into 15 bivalents including the sex (*X* and *Y*) chromosomes (Figure 3). Comparison of the karyotypes of the 2 species *M. dobsoni* and *M. talazaci* show obvious variation in 2 chromosome pairs. The largest pair in *M. dobsoni* is an acrocentric chromosome (labelled as No. 1 in the karyotype). This pair appears to exist also in other species of the sub-family Tenrecinae[3]: *Centetes ecaudatus* Schreber[4], *Hemicentetes semispinosus* Cuvier, *H. nigriceps* Gunther, *Setifer setosus* Froriep, *Echinops telfairi* Martin, whereas the largest pair in *M. talazaci* (labelled as No. 1 in the karyotype) is sub-metacentric. This sub-metacentric chromosome is longer than the largest acrocentric pair of *M. dobsoni* and in fact the long arms of the acrocentric pair of *dobsoni* and the sub-metacentric pair of *talazaci* are of equal length. The species also differ in that there are 2 small acrocentric chromosome pairs in *dobsoni* (probably 10 and 13) and 3 in *talazaci* karyotype (probably 9, 10, and 13). We postulate that the short arm of the long sub-metacentric chromosome in the *talazaci* karyotype (No. 1) has been translocated to the medium sized acrocentric chromosome in the *talazaci* karyotype (probably No. 9) giving rise to a metacentric chromosome (probably No. 9) and the large acrocentric chromosome (No. 1) in the *dobsoni* karyotype. A diagrammatic illustration of the possible mechanism is illustrated in Figure 4. Measurements of the chromosome lengths and arm ratios confirm this interpretation. If the hypothesis of interchange of segments between these 2 chromosome pairs is correct then this may have been the cause of variation in the animals. Interchange of chromosome segments would change gene combination and might exhibit position effects[7]. In heterozygous condition translocations would give rise to partial

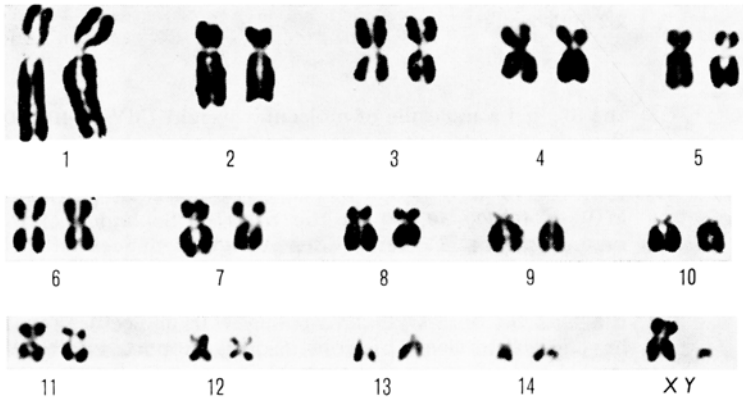

Fig. 1. A karyotype of a male *Microgale talazaci*.

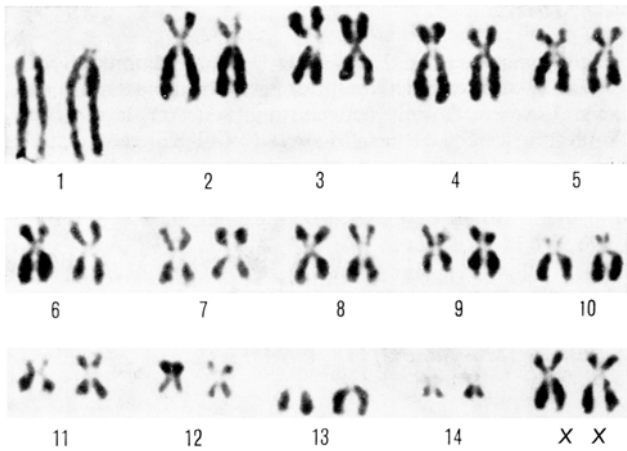

Fig. 2. A karyotype of a female *Microgale dobsoni*.

[1] O. THOMAS, A. Mag. Nat. Hist., London *1*, 303 (1918).
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[7] TH. DOBZHANSKY, *Genetics and the Origin of Species* (Columbia University Press, New York 1941).

List of animals studied, chromosome counts, and locality where collected

Species	No.	Sex	Lab. No.	Tissue	Counts					Meiosis			Locality
					28	29	30	Endo	4N	30 II	15 II	14 II 2 I	
M. talazaci	3	F	1536	Blood	Failure								Perinet (18° 54′ S, 48° 24′ E)
				Spleen	Failure								
				Skin SC 2	0	1	6		1				
				SC 3	0	2	3						
M. talazaci	4	M	1537	Blood	Failure								
				Spleen			7	2					
M. talazaci	5	M	1543	Blood			1						
				Spleen	1		1		2				
M. talazaci	6	F	1544	Spleen	Failure (contaminated)								
M. talazaci	7	M	1545	Spleen			16		1				
				Blood			4						
M. talazaci	8	F	1546	Spleen			13						
				Blood	Failure								
M. talazaci	9	M	1551	Blood	Failure								
				Spleen			11						
					1 break				3				
M. talazaci	10	M	1558	Blood	Failure								
				Spleen			2						
				Testis			1		1		12	1	
M. talazaci	11	M	1614	Blood	Failure								
				Spleen		2	10		2				
M. talazaci	12	M	1617	Testis			6			3	12	2	
				Spleen	4	1	10						
M. dobsonii	1	M	1655	Spleen			4						Manandroy (21° 9′ S, 47° 15′ E)
M. dobsonii	3	F	1575	Skin SC 2		1	15		12				Manohilahy, 22 km W of Morano-Sud (19° 17′ S, 48° 01′ E)
				Spleen	Spleen		4						
M. dobsonii	4	F	1576	Blood	Failure								
				Spleen	Failure								
				Skin SC 2a		1	11		2				
				SC 2b	10	5	17	3	6				
M. dobsonii	5	F	1616	Spleen		1	7						
M. dobsonii	6	F	1630	Spleen			9						
M. dobsonii	7	F	1631	Spleen		1	6	3					

sterility and inviable offspring[8]. Once an interchange becomes homozygous it may become advantageous in a particular environment[7]. That chromosomal rearrangements such as these do cause variation in organisms is well known[7,8]. Further, we believe that such variation here has led to speciation.

Morphologically the 2 taxa *M. dobsoni* and *M. talazaci* can be separated, among other characters, on the basis of the length of the head, body and tail, skull characteristics, and fattening of the tail[9]. Measurements on our animals enable us to group them in these 2 taxa and karyotype data support the identification, the 2 approaches having been applied independently. The specimens will be deposited with zoological museums.

Homozygous reciprocal translocations in these 2 species of *Microgale* could be confirmed by obtaining a hybrid between the 2 species. Meiotic study of such hybrid animals should reveal a quadrivalent association of 4 chromosomes because of homology of segments of the 4 chromosomes involved in this rearrangement. These species are difficult to breed in captivity and attempts are being made to obtain progeny.

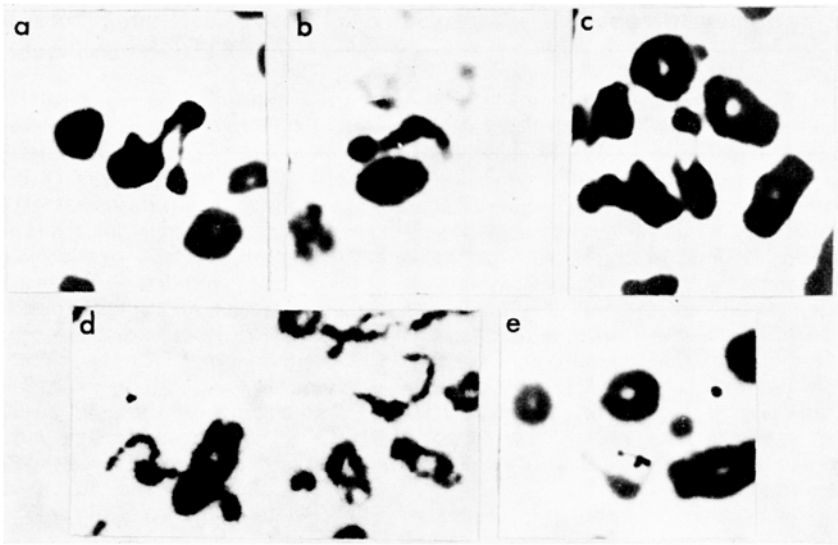
Homozygous reciprocal translocation is not considered to be a common mechanism of speciation[10]. It is, however, known to occur in experimental populations, barley[11];

[8] C. P. Swanson, *Cytology and Cytogenetics* (Prentice-Hall, Inc., Englewood Cliffs, New Jersey 1957).

[9] T. C. S. Morrison-Scott, Proc. Zool. Soc. Lond. *118*, 817 (1948–49).

[10] E. Mayr, *Animal Species and Evolution* (Harvard University Press, Cambridge 1963).

[11] A. Hagburg and J. H. Tjio, An. Estac. exp. Aula Dei *2*, 215 (1952).



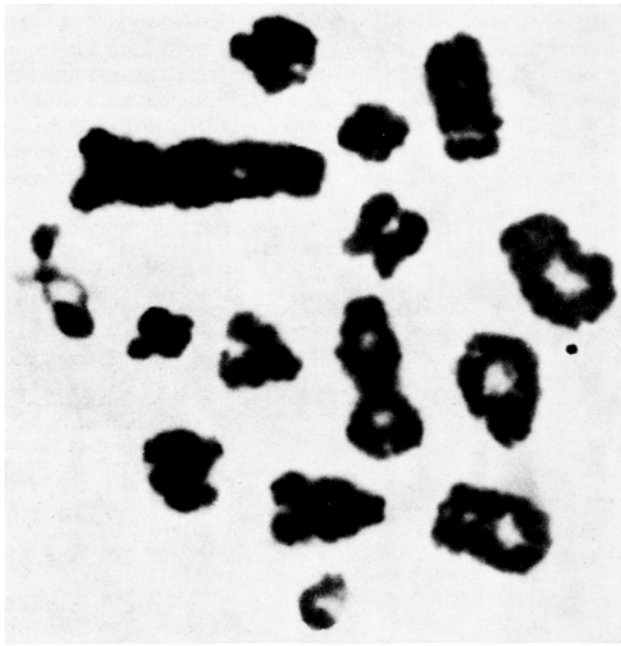


Fig. 3. (A) A plate showing the XY bivalents from different cells. (B) A first meiotic metaphase cell showing 15 bivalents.

Drosophila[12]. If one considers Robertsonian[13] fusion of acrocentric chromosomes as a kind of reciprocal translocation then probably at least for the resulting metacentric chromosome it is similar to the hypothesis reported here. BURNHAM[14] states that a survey of interchanges in geographic races shows that they are relatively rarely established in nature and are known in maize[15]; Datura[16]. Translocation heterozygotes are known to occur in wild popula-

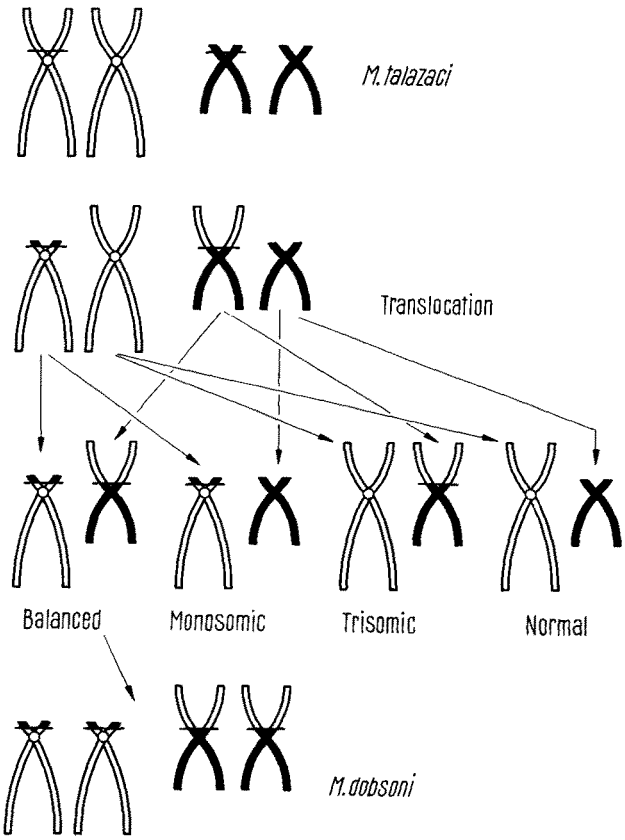


Fig. 4. A diagrammatic illustration of the mechanism of breakage and reunion of the chromosome pair in *Microgale* species and its fixation in a homozygous condition.

[12] C. B. BRIDGES and K. S. BREHME, *The Mutants of Drosophila melanogaster* (Carnegie Inst. Wash. Publ. *552*, Washington D.C. 1944).
[13] W. R. B. ROBERTSON, J. Morph. *27*, 179 (1916).
[14] C. R. BURNHAM, *Discussions in Cytogenetics* (Burgess Publishing Company, Minneapolis 1962).
[15] D. C. COOPER and R. A. BRINK, Am. Nat. *71*, 582 (1937).
[16] A. F. BLAKESLEE, A. D. BERGNER and A. G. AVERY, Cytologia Fujii Jub. vol. 1070 (1937).

tions in grasshoppers[17], beetles[18], *Drosophila ananasse*[19]. However, some authors in the past[20] have concluded that there is no direct correlation between translocations and speciation.

We believe that the present study illustrates very well the karyotype variation between the 2 species of *Microgale*, *M. dobsoni* and *M. talazaci* and that the mechanism by which such a variation might have occurred in all probability is reciprocal interchange of chromosome segments[21, 22].

Résumé. Deux espèces d'insectivores, *Microgale dobsoni* et *M. talazaci*, ont le même nombre de chromosomes, $2n = 30$. Leurs caryotypes respectifs présentent des différences portant sur 2 pairs d'autosomes. Une translocation réciproque, devenue homocygote, permet d'expliquer ces différences. On peut concevoir que la spéciation est alors intervenue à la suite d'effets de position, de la stérilité des hybrides et de la préférence des porteurs de formules chromosomiques différentes pour telle ou telle «niche» écologique.

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[21] Acknowledgements. The authors gratefully acknowledge the generous hospitality and help given by Drs. Brygoo, Dodin, Mayeaux of the Institut Pasteur, Tananarive, Madagascar, and thank Dr. J. Eisenberg for some useful suggestions and discussion. Expert technical assistance of H. Herr, E. Mules, and K. Johnson in the chromosome laboratory is appreciated.
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On the Genetics of a Circadian Rhythm in *Drosophila*

From all we know so far certain DNA-controlled processes seem to play an important part in the control of cellular circadian rhythms[1]. It is, however, not clear whether the entire genome, chromosome sections, or single genes determine the parameters of the cellular rhythm and maintain the oscillations. Probing experiments had led us to the idea that sexual differences in the circadian rhythm may be correlated to the ratio of X chromosomes to autosomes. In order to analyze this question in more detail we tested the pattern of the circadian rhythm of oxygen consumption in 26 mutants of *Drosophila melanogaster* Meigen.

Each experiment was started when the flies were 3–6 days old. O_2-consumption was measured for groups of 5 animals and for single controls in 12 ml and 6 ml Warburg flasks, respectively. The water bath around the flasks was kept at 25° ± 0.01 °C. The flies were exposed to light (90 lx) from 09.00 to 21.00 h. Readings from 3 flasks (with 5 animals each) were averaged over 2 days and the standard error calculated. In our method the absolute values may depend on the diffusion- and absorption-velocities of the CO_2 emitted. For the present argument, however, only relative changes are considered.

The results of the various mutant stocks indicate that the circadian pattern of oxygen consumption of females (Figure 1, b, c, d) and males (Figure 1f, g, h) differ. As shown previously[2], wild type females and males show a morning and an evening peak of oxygen consumption (Figure 1d, f), a pattern that also appears in the hormonal system[3]. In females, however, the morning maximum is smaller than the evening maximum, and in males both maxima are almost equal with respect to the total oxygen consumed between 2 minima. In about half of the mutants tested, the females show only a single peak in the evening (Figure 1b), or a second peak in the morning is just faintly indicated, e.g. in a triploid stock (Figure 1c). The males never have only one peak in the evening. They show, varying in extent, higher morning and lower evening maxima (Figure 1f, g, h), as compared to the females. The closed-X mutants, in particular, yield a very small evening maximum or, in some experiments, none at all.

If these differences in the circadian pattern were due to a different ratio of X chromosomes to autosomes it should be possible to support this idea by testing other ratios, as represented e.g. by intersexes (0.67) or superfemales (1.5). Intersexes of the triploid stock (Figure 1e) show, in fact, a morning maximum that is about the intermediate of the females (Figure 1c) and the males of this stock. (The additional broadening of this peak into the dark period is probably connected with the gene w^a, since w-males also show this change.) Four females, out of approximately 900 females of an attached-X stock (cs^{53}/*y w bb*) which showed wild phenotype and turned out to be sterile, we assumed to be superfemales. In one case we observed a detachment in this stock, where the appropriate number of males with *y w bb* turned up in the progeny. The attached-X and the detached-X females and the males show a two-peak pattern of O_2-consumption, whereas the 4 superfemales tested exhibit a pronounced, single maximum in the evening (Figure 1a).

If one arranges the curves according to a relative decrease in the evening maximum and to a relative increase in the morning maximum, it becomes evident that in this sequence also the ratio of X chromosomes to autosomes

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