A COMPARATIVE CHROMOSOME STUDY OF RATTUS RATTUS MINDANENSIS AND RATTUS ARGENTIVENTER[1]

DORIS H. WURSTER and GERRY C. ATWELL[2]

Rattus argentiventer and *Rattus rattus mindanensis* are important agricultural pest species in the Philippines. Comparative chromosome studies have been performed on these species to further clarify their taxonomic status and gain insight into their cytogenic relationship. The two forms can be consistently identified by differences in their sex chromosomes.

Rodents of the genus *Rattus* have long been a problem in agricultural areas of the Philippines. Damage to rice, corn, coconuts, sweet potatoes, bananas, sugar cane, peanuts, cantelope and watermelons is extensive. Biologists at the Rodent Research Center at the University of the Philippines' College of Agriculture estimated the preharvest loss of rice due to rats at $8,000,000 (Swink et al., 1971). This estimate was based on the nationwide appraisal of rat damage to rice conducted by the Bureau of Plant Industry in 1970. Losses of other crops have not been quantitatively documented but can be extremely high at the local level.

Through the late 1960's, investigators working in lowland areas in the Philippines did not agree on the taxonomy of the "ricefield" rat. Usually the taxa *Rattus rattus umbriventer* Kellogg, *R. r. mindanensis* Mearns and *R. r. argentiventer* Robinson and Kloss were used synonymously (Sumangil, 1963; 1965). Clark (1968) indicated that at least two of these animals were indeed true subspecies. Based on specimens identified at the British Museum he stated that in Cotabato, the most important pest species was not *R. r. mindanensis*, "but a subspecies with a relatively shorter tail and darker, grayer, coarser fur". This was *Rattus r. argentiventer.*

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[1] General Contribution No. 1039

[2] Respectively, staff of the Department of Pathoogy, Dartmouth Medical School, Hanover, N.H. 03755, and Research Biologist, Kodiak National Wildlife Refuge, Box 825, Kodiak, Alaska 99615.

In 1969 the first nationwide distribution study of ricefield rats was conducted by Barbehenn et al. (1972). From this extensive collection it was determined that the two most economically important ricefield rats were *R. r. argentiventer* and *R r. mindanensis*. Barbehenn (personal communication) concurs with Harrison (1961) that *R. argentiventer* should be given full species recognition. Biologists at the Rodent Research Center now adhere to this usage.

Ecological and toxicological research for control methodology must be geared to the lowest taxon for meaningful results. Subtle behavioral or physiological differences among target organisms may mean success or failure in a control operation. To gain further insight into the differences between *R. argentiventer* and *R. r. mindanensis* it was decided to compare them genetically through karyotyping.

MATERIALS AND METHODS

Specimens of *R. argentiventer* were live-trapped in Mindoro, one of the two Philippine islands where they are found, the other being Mindanao (Barbehenn et al., 1972). Specimens of *R. r. mindanensis*, which is found throughout the Philippines, were live-trapped in Luzon.

Full-thickness skin biopsies were taken from freshly captured animals and placed in McCoy's 5ª medium (modified) fortified with 20% fetal calf serum for shipment to the United States. Fibroblast cultures were established in large Leighton tubes and fed with the same medium used for shipment. When growth was sufficient, subcultures were established and harvested at a time of good mitotic activity. The cells were removed from the flask with 0.25% trypsin and washed twice with Earle's balanced salt solution. They were treated for 13 minutes in a hypotonic solution of 1 part Earle's solution to 5 parts distilled water, and then fixed in acetic acid:methanol (1:3). After 3 washes in fixative, the cells were placed on slides, air-dried and stained with carbol-fuchsin stain (Carr & Walker, 1961). Chromosomes were photographed with a Zeiss photomicroscope at a magnification of 400 X. For each specimen a minimum of 20 cells was counted and 5-15 karyotypes were prepared. Four male and 3 female *Rattus argentiventer* and 7 male and 2 female *R. r. mindanensis* were studied. One male *R. argentiventer* from South Vietnam was studied for comparison.

RESULTS

The diploid chromosome number of both *Rattus argentiventer* and *Rattus rattus mindanensis* is 42. Each species has a fundamental number (total number of major chromosomal arms) of 60. The autosomal complements of the two species are identical in appearance, each being composed of 1 pair of large subacrocentric chromosomes, 1 pair of large, 4 pairs of medium-sized and 6 pairs of small size-graded acrocentric chromosomes, and 7 pairs of small metacentric and submetacentric chromosomes. The sex chromosomes of the two species differ consistently in size. The X chromosomes of *R. argentiventer* is acrocentric and is the sixth or seventh largest element of the haploid complement; the Y chromosome is also acrocentric and is usually the third in size from the smallest acrocentric autosome (Fig. 1). The X chromosome of *R. r. mindanensis* is acrocentric and is the third largest element of the haploid complement; the Y chromosome is acrocentric and is the smallest element of the complement (Fig. 2). The karyotype of the *R. argentiventer* specimen from South Vietnam was identical in every way to those of the *argentiventer* specimens captured in the Philippines

DISCUSSION

The taxonomy of the many forms of the huge genus *Rattus* is confused. Morris (1965) states that there are 137 recognized species of *Rattus,* and many more forms than this are named. A comprehensive karyological study of 18 species of Malayan rats has been made by Yong (1969[a]), who presented karyotypes of species in each of the five Malayan subgenera of *Rattus* recognized by Ellerman and Morrison-Scott (1955). Yong's study based on chromosomal evidence indicated that the component species of three of these subgenera could be considered to be closely related, while the component species of the other two subgenera are probably more distantly related. Yong's studies (1969[a], 1969[b]) are the first on the genus *Rattus* that are comprehensive enough to indicate the use of karyological studies as a useful method in the taxonomic clarification of a confused genus. Yosida et al. (1969[a], 1969[b]) have also contributed substantially to chromosomal studies of this genus in Southeast Asia. Duncan et al. (1970; 1971), Capanna et al. (1970) and Bianchi et al. (1969) have each studied some *Rattus* forms in Southeast Asia, Europe and South America, respectively. Badr and Badr (1970) have studied a wild population of *Rattus rattus* in Egypt. Ray-Chaudhuri and Pathak (1970) have also informally reported chromosome studies in some species of *Rattus* from India,

and Markvong (1972) has similarly reported on a number of species from Thailand. It is not our purpose here to review these studies, but to point out that karyological investigation is gradually being applied to help resolve the taxonomic mysteries of the large genus *Rattus*.

In the present study, specimens of *R. argentiventer* and *R. r. mindanensis* were collected for comparative karyotype studies. The goal of this investigation was the clarification of the status of each form, i.e., to determine whether the terms *R. r. argentiventer* and *R. r. mindanensis* might be used synonymously (Sumañgil, 1963 and 1965); the alternatives to consider were that the two forms are true subspecies, or that *argentiventer* should be given full species recognition.

The use of comparative karyology in taxonomy lies only in positive results within well-defined taxonomic groups. Two species may have similar karyotypes and be unrelated. For example, the lesser panda (*Ailurus fulgens*) has a karyotype that is similar to that of the clouded leopard (*Neofelis nebulosa*) (Wurster, 1969), but this does not necessarily indicate any relationship. On the other hand, two separate but related species within one genus may have identical karyotypes, e. g., Indian golden cat (*Felis temmincki*) and serval (*Felis serval*) (Wurster and Benirchke, 1968). In this case, one may only say that on karyotypic evidence alone one could not separate the forms into two distinct species. If, however, two separate forms within a genus consistently have distinct karyotypes by which they can be separately identified, one may say that there is a karyotypic basis on which to distinguish these forms into two different categories.

Both structural and numerical polymorphism have been reported for *Rattus rattus* and *R. norvegicus* (Badr and Badr, 1970; Gropp *et al.*, 1970 Yosida et al., 1965; Yosida and Amano, 1965; Yosida, 1968; 1971). The occurrence of polymorphism must be considered when comparing karyotypes from different *Rattus* species, and this necessitates the study of at least several specimens of each form to establish consistent features.

Yong (1969a) studied one male and two female specimens of *R. argentiventer* from Malaysia. The autosomal complement of this male specimen was similar to ours, but the sex chromosomes differed in that the X was subterminal (subacrocentric by our term) and the Y was the smallest element in the complement. Since the single studied specimen of *R. argentiventer* from South Vietnam (Duncan and Van Peenen, 1971) had a karyotype identical to those of Philippine species, we must conclude that the Malaysian specimens of *argentiventer* are different forms of *Rattus* from the *argentiventer* specimens studied. Both *argentiventer* and *minda-*

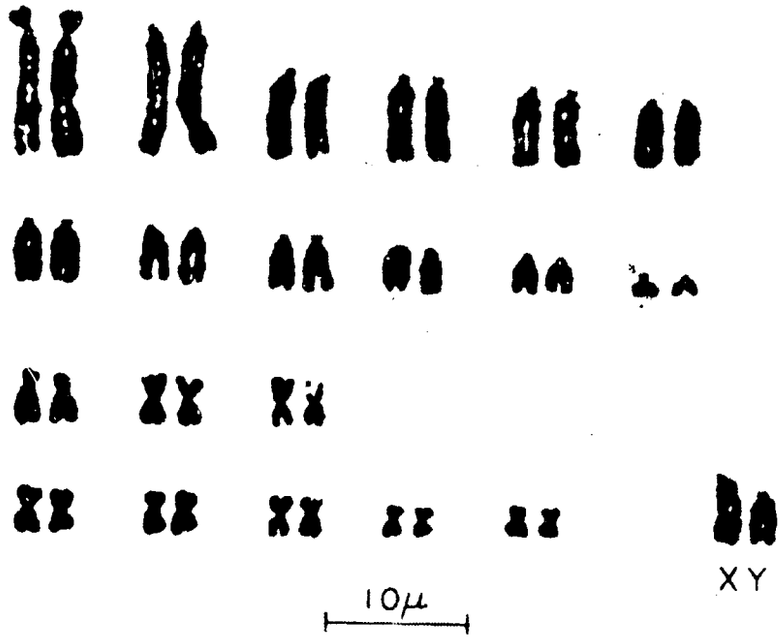

Figure 1. Karyotype of a male Rattus argentiventer. 2000 X.

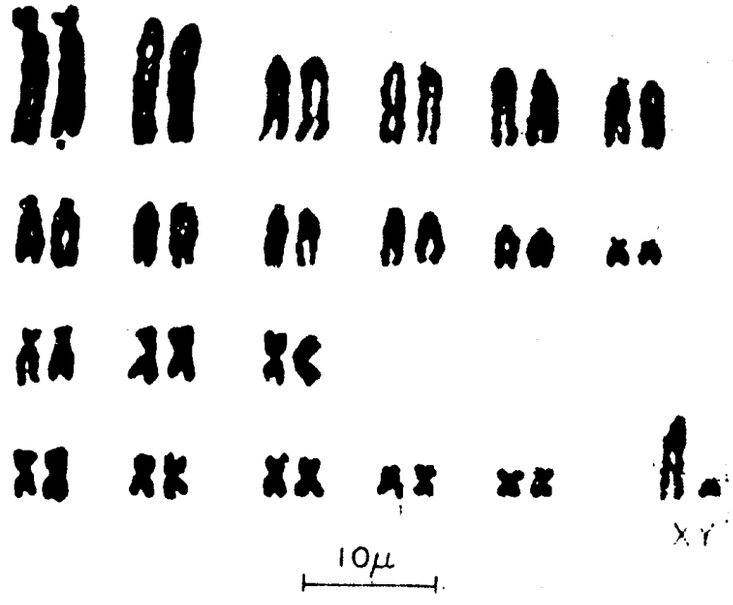

Figure 2. Karyotype of a male Rattus rattus mindanensis. 2000 X.

nensis have been studied by Yosida *et al.* (1971) and reported as subspecies of *Rattus rattus* with similar karyotypes.

This study has shown that the two forms of *Rattus* under investigation have karyotypes that can be distinguished on the basis of the sex chromosomes, the Y chromosome in particular. Since nine *mindanensis* and seven *argentiventer* were studied, it can be stated with reasonable certainty that the difference between the karyotypes is consistent. Thus we conclude that there is a karyotypic basis on which to place these two forms into separate categories, and karyotypic evidence that the terms *Rattus rattus mindanensis* and *Rattus rattus argentiventer* may not be used synonymously. One could not, solely on the basis of karyotype, confer on *argentiventer* a full species recognition. The biological evidence, however, strengthens the position of classical taxonomists who, on other grounds, feel that *argentiventer* is a separate species.

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