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THE CHROMOSOMES OF *APANTESIS PHALERATA, A. RADIANS,* AND THEIR HYBRID IN FLORIDA POPULATIONS (ARCTIIDAE)[1]

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OCURRING WIDELY in the southeastern portion of North America are two very similar and commonly confused species of *Apantesis,* or "tiger moths." *Apantesis phalerata* (Harris), a more broadly distributed moth, has recently been shown in northern Florida populations (Bacheler, 1972) to be specifically distinct from *A. radians* Walker in several biological and morphological characters. The adults of these two species and their hybrid are illustrated in Fig. 1. We wish to report here the surprising differences in chromosome numbers between the two closely related species, and their experimentally-produced hybrids. These chromosome counts represent the first published data for species in this arctiid genus.

METHODS

Meiotic divisions in the Lepidoptera are most easily observed in testes of male adults (Emmel, 1969) but spermatogenesis was essentially completed in almost all *Apantesis* adults examined. Further preliminary studies showed that germ cell division in *A. phalerata* and *A. radians* occurred to the greatest degree during the penultimate instar of male larvae. Thus the standard procedure for chromosome preparations previously reported (Emmel, 1969) was modified as follows.

Testicular tissues of late penultimate *A. phalerata, A. radians,* and *A. phalerata* ♀ X *A. radians* ♂ hybrid larvae were fixed by injecting a 3:1 absolute ethyl alcohol : glacial acetic acid mixture into the sixth abdominal segment with a no. 27 hypodermic syringe. The injected larvae were placed into four ounce jars containing the same fixative, labeled, and refrigerated at about —20°C for later dissection.

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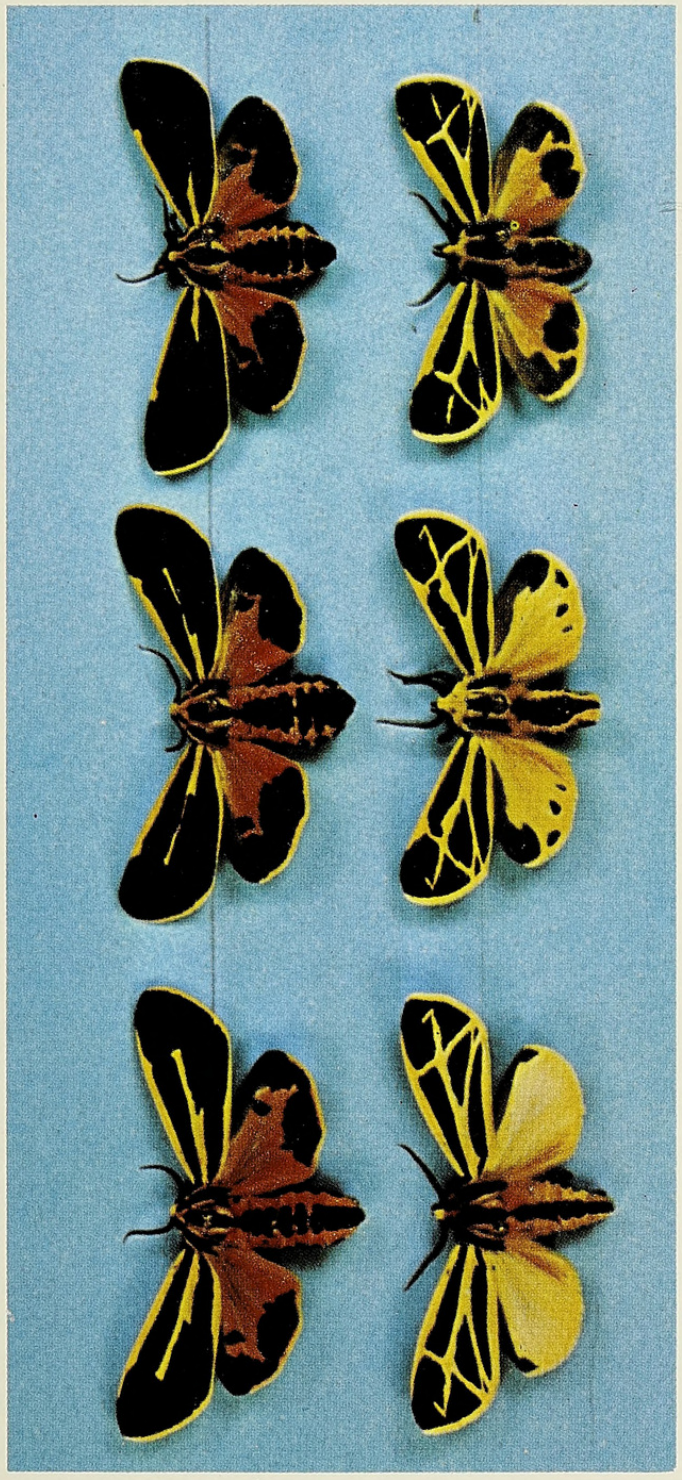

Fig. 1.—Males (lower specimens) and females of *Apantesis phalerata* (left), *A radians* (right), and their hybrid (center).

For chromosome study the testes, usually found in the dorsal area of the sixth abdominal segment of male larvae, were removed with No. 5 watchmaker's forceps and placed onto a standard microscope slide. The testes were then macerated and a few drops of Lacto-Aceto-Orcein Stain (Emmel, 1969) added. This preparation was allowed to stand for about 10-12 min. The stained testes were next covered with a coverslip and the preparation squashed with about 400 lb/in.^{2} pressure between two pieces of blotting paper. The perimeter of the coverslip was then sealed with clear lacquer.

Slides were inspected with a Zeiss Research Microscope STANDARD WL fitted with 25X and 40X plan-apochromatic field objectives. An oil-immersion Planapo 100X objective was used for critical observations.

Chromosome counts were made during meiotic division I when chromosomes were paired in synapsis. Photographs were taken of unusually clear chromosomes sets and of any interesting anomalies.

All larval material was reared in the laboratory from wild females of both species collected in populations in the vicinity of Gainesville, Alachua County, Florida. Hybrid larvae were obtained from laboratory crosses of virgin adults of the two species.

RESULTS AND DISCUSSION

Definitive chromosome counts with photographic confirmation were obtained from microscopic slide examination of *phalerata, radians,* and hybrid larval testes squashes. The apparent haploid chromosome numbers of *phalerata, radians,* and the hybrid cross are n = 29, 30, and 29 respectively (Fig. 2). Since no other counts have been reported in the genus the interpretation of the chromosomal evidence for the phylogenetic relationship between these two species is necessarily limited and speculative. However, there are two principal ways in which a difference in lepidopteran chromosome numbers of this type may be explained and some evidence exists to indicate which hypothesis is more tenable.

1. *Reduction in Number by Fusion or Loss*

The karyotypic origin of *phalerata* (n = 29) could have resulted from the fusion of two of *radians'* (or an ancestor of *radians*) 30 chromosomes, or a loss of one ancestral chromosome. A reduction in chromosome number from a characteristic

lepidopteran haploid set of 31 is not infrequent in the arctiids and closely related noctuids; in counted species of both families the mean haploid number is 31 chromosomes (Robinson, 1971). The reported arctiid counts show one species with a haploid number of 26, four with 28, one with 29, two with 30, seventeen with 31, and one species with a number varying between 30 and 33. The noctuids show the same apparent tendency toward reduction in chromosome number for the probable ancestral number of 31, with seven species having less than 31 and three exceeding 31.

Thus it is possible that *radians* resulted from the fusion of two chromosomes in an n = 31 ancestor, and that *phalerata* was the result of a further fusion of two *radians* chromosomes. These fusions would reduce slightly the amount of possible genetic recombination and thus the general adaptive flexibility of the species. But fusion would be an evolutionarily favorable event for a well adapted species where the need for genetic recombination would be minimally valuable, even selectively disadvantageous.

If *phalerata* had indeed evolved with fusion from an ancestor with a haploid number of 30, such as *radians*, microscopic examination of its 29 chromosomes might show one double-sized or exceptionally large chromosome. However, photographic examination did not disclose a significantly larger chromosome. Thus it seems more probable that loss of a chromosome from the *radians* complement or loss of two chromosomes from a common ancestral complement represents a plausible explanation of the origin of *phalerata's* n = 29 set of chromosomes.

2. *Hypothesis Involving Increase in Number by Fission (Fragmentation)*

The possibility also exists that *phalerata* (or an n = 29 ancestor of *phalerata*) gave rise to *radians* (or an ancestor of *radians*) by fragmentation of one of its chromosomes. The evolution of many lepidopteran species has been accompanied by an increase in chromosome number (Emmel, 1972; Emmel and Trew, 1973). Thus, one of *phalerata's* 29 chromosomes could have split, with both parts being retained in the new genotype, resulting in a new chromosome complement of 30. The selective advantage of this fragmentation would be increased potential for genetic recombination, allowing the species to be more generally adaptable. (Naturally, the ancestral number of 29 in *phalerata* would likely have been derived originally by reduction through loss or fusion.)

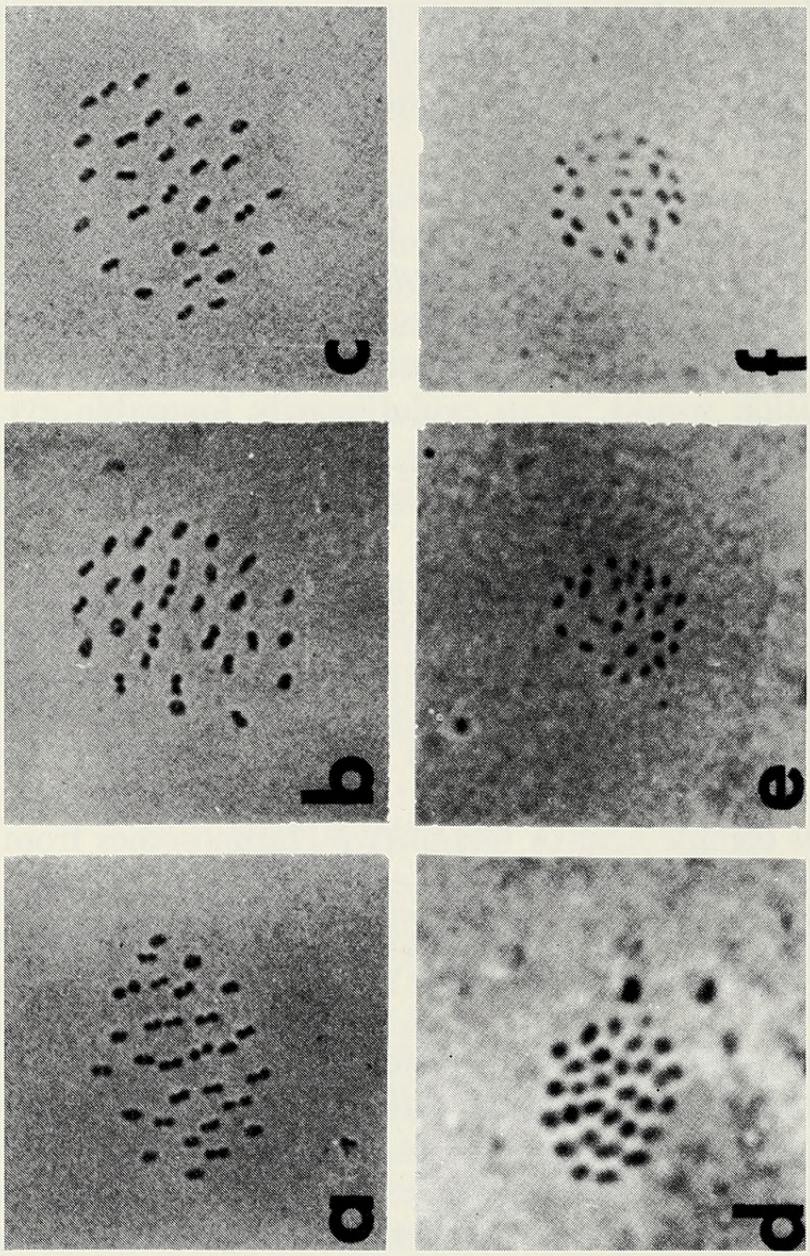

Fig. 2.—Photomicrographs of testes squashes at 1110X magnification; chromosomes in metaphase during first meiotic division. (a-c) *Apantesis phalerata*, F_1 brood L-60 (n = 29); (d) *A. radians*, F_1 brood D-62 (n = 30); (e,f) *A. radians* ♂ x *A. phalerata* ♀, brood DL-6261 (n = 29).

There is some support for the above fragmentation hypothesis in *phalerata* and *radians*. If *radians*' karyotype had resulted from the fragmentation of one of the ancestral *phalerata* (or similar n = 29 species) chromosomes, two smaller chromosomes might be found among the 30. Two *radians* chromosomes (Fig. 2d) do appear smaller than the others.

If *radians* had evolved from *phalerata*, one probably would expect *radians*' range to be more restricted, having split off rather recently (suggested by their almost identical morphology, life history, and occasional laboratory hybridization). Such is the case, with *radians* occupying about a third the area that *phalerata* does.

EVIDENCE FROM THE HYBRIDS OF THE TWO SPECIES

If one accepts the ancestral fragmentation hypothesis as more likely, the hybrid chromosome number of 29 is most easily explained by the two short chromosomes of the *radians* parent pairing with their "former" chromosome from the *phalerata* parent in the hybrid larvae testes (at meiosis I). A uniform count of 29 in meiosis II cells of the hybrid larvae would result from the loss of one of the two small chromosomes after pairing in meiosis I (the loss of both small chromosomes would have meant the probable additional loss of the unpaired *phalerata* chromosome, resulting in a hybrid count of 28, or at least a variable count of 28 and 29 in meiotic (II) cells of the hybrid adult).

If one follows the ancestral fusion hypothesis (Section I), the picture in the hybrid would be superficially similar (n = 29 at meiosis I), only the two complete chromosomes of the *radians* parent would pair with the large, "fused" chromosome of the derivative *phalerata* parent in the hybrid larval testes cells. The testes of such a hybrid male larva should have a variable number of chromosomes (28 or 29) in meiotic cells, or if all cells were n = 29 (due to the loss of just one *radians* parental chromosome), about half the cells in meiosis II should have one chromosome noticeably smaller than the other 28 and half the cells should have all 29 chromosomes appearing as uniformly-sized.

Unfortunately, our material did not provide sufficient plates of material in meiosis II to ascertain whether such a chromosomal loss was indeed the case.

SUMMARY

Apantesis phalerata and *A. radians* are two closely related, phenotypically similar arctiid species found sympatrically in northern Florida. Examination of meiotic divisions in testes of penultimate instar larvae indicated that the haploid chromosome numbers for the two species are different: n = 29 for *phalerata* and n = 30 for *radians*. Their hybrid has a chromosome number of n = 29. These are the first cytological observations reported from the genus *Apantesis*.

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LITERATURE CITED

BACHELER, JACK S. 1972. Biology and hybridization of *Apantesis phalerata* (Harris) and *A. radians* Walker in Florida (Lepidoptera: Arctiidae). Department of Entomology and Nematology, University of Florida, Ph.D. thesis. 89 pp.

EMMEL, THOMAS C. 1969. Methods for studying the chromosomes of Lepidoptera. *Journ. Res. Lepid.* 7: 23-28 ("1968").

———. 1972. Mate selection and balanced polymorphism in the tropical nymphalid butterfly, *Anartia fatima*. *Evolution* 26: 96-107.

EMMEL, THOMAS C., and HOUSTON R. TREW. 1973. The chromosomes of skipper butterflies from southwestern North America (Lepidoptera, Hesperiidae). *Cytologica,* 38: 45-53.

ROBINSON, ROY. 1971. Lepidoptera Genetics. Pergamon Press, Oxford. 687 pp.