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NATURAL HYBRIDIZATION IN THE COCCINELLID GENUS CHILOCORUS*

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Abstract. A population of lady-bird beetles at the head of the Crowsnest River valley on the immediate east side of the Rocky Mountains has been sampled regularly over the years 1961 to 1964. The 389 individuals studied had chromosome numbers ranging from $2n = 14$ (58 %) to $2n = 20$ (3 %). The former is the diploid number of *C. hexacyclus*, the latter that of *C. tricyclus*, species otherwise known respectively only in the Prairie Provinces and the interior of British Columbia plus the adjoining part of the State of Washington. About 19 % of the beetles had the intermediate number of 17 chromosomes, males possessing either a *hexacyclus*-type or a morphologically distinct *tricyclus*-type Y chromosome. — In all numerical categories meiotic pairing was virtually perfect but the trivalents in heterozygotes were frequently linearly aligned. This assures production of aneuploid (duplication-deficiency) gametes, only complementary recombinations of which are viable. Segregational sterility resulting from triple (92 %), double (84 %) and single (50 %) heterozygotes is estimated to be *ca* 40 %. Of four neo-diploids, with zero chromosomal sterility, predicted in the F_2 only one materialized with certainty, the one with 13 + *tricyclus*-type Y. This is interpreted to mean that F_2 progeny are rare; rare because (1) random mating, complete introgression, is the rule and (2) segregational sterility is severest in triple heterozygotes. — Despite the population-depressing effect of the inviable aneuploid zygotes, it is calculated that segregation and crossing-over generate a minimum of 148×10^9 new recombinants (a minimum were crossing-over *strictly* localized); those acquiring beneficial attributes will be favoured by natural selection. — Topographically, it is evident that *tricyclus* adults invade the resident *hexacyclus* community year after year, borne on the prevailing westerly winds that funnel through Crowsnest Pass (elevation only 4463′): *hexacyclus* is thereby constrained to the east of the Continental Divide and consequently does not penetrate into *tricyclus* territory.

Introduction

Introgressive hybridization, the feeding-back of alien genes from F_1 hybrids and hybrid derivatives into their parental species, has not been widely studied in animals, particularly not at meiosis. Conclusions favouring its occurrence are mostly founded on comparative *external* morphology: since parallel selection of similar gene alleles would provide the same end-results, such conclusions are open to question. On the other hand, WHITE and KEY's (1957) arguments against introgression as the cause of overlapping phenotypes in Australian *Austroicetes* grasshoppers carry conviction because they are based on comparative *internal* mor-

* Dedicated to Prof. J. SEILER on the occasion of his eightieth birthday.

phology—on cytological analysis: the two supposedly interbreeding species were shown to differ in several chromosomal diagnostics, to the complete absence of intermediates. Cytological examination of population samples of the twice-stabbed lady-bird beetle taken on the immediate east side of Crowsnest Pass through the Rocky Mountains has, on the contrary, provided unambiguous proof for the occurrence of both natural hybridization and subsequent introgression.

In Canada, this common name, the twice-stabbed lady-bird beetle, is unwittingly applied to three species in the genus *Chilocorus*, namely, *C. stigma* SAY (=*bivulnerus* MULSANT), *C. hexacyclus* SMITH and *C. tricyclus* SMITH. The three are superficially indistinguishable but have different overall distributions and chromosome numbers; *stigma* ranges from Nova Scotia to north-western Alberta (SMITH, 1962); *hexacyclus* is found only in Saskatchewan and southern Alberta; *tricyclus* is, so far as known and except as will be mentioned, confined to the interior of British Columbia and at least the adjoining portion of the State of Washington (SMITH, 1959, and unpub.).

Despite the apparent absence of external differences, the three species are readily categorized chromosomally and it is upon this basis that the fact of introgressive hybridization can be so rigorously documented.

Material and Methods

Samples of *Chilocorus* from just east of Crowsnest Pass were received in 1957, 1961, 1962 and 1963 through the co-operation of divisional field staff working out

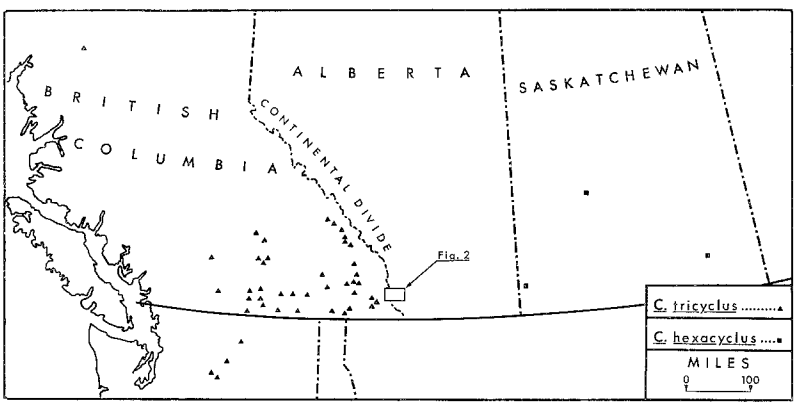


Fig. 1. The known distribution of *C. tricyclus* and *C. hexacyclus*. Note that *hexacyclus* is confined to the east and *tricyclus*, except for the area of overlap, to the west of the Continental Divide, the ridge of the Rocky Mountains

of the Calgary, Alberta, Forest Biology Laboratory. In 1964, larger samples were collected in person with the invaluable assistance and guidance of Mr. J. PETTY.

At an altitude of 4,463 feet above sea level, Crowsnest Pass through the Rocky Mountains stands at the head of the Crowsnest River vally (Fig. 2), the river itself

flowing generally eastward through the foothills into the Alberta prairies and eventually into the South Saskatchewan River. The pass is flanked to the north by the High Rocky Range and to the south by the Flathead Range, the former having minimum elevations of over 5,000 feet, the latter being mostly over 6,000 feet. The foothills in the sampling area support coniferous trees dwarfed and gnarled by the often severe, bitterly cold prevailing winds that funnel through the pass from the west. Over the years of study these trees have been infested by the pine-needle scale, *Phenacaspis pinifoliae* (FITCH), on which *Chilocorus* feeds. Conifers, unless planted, are absent from the Prairie grasslands.

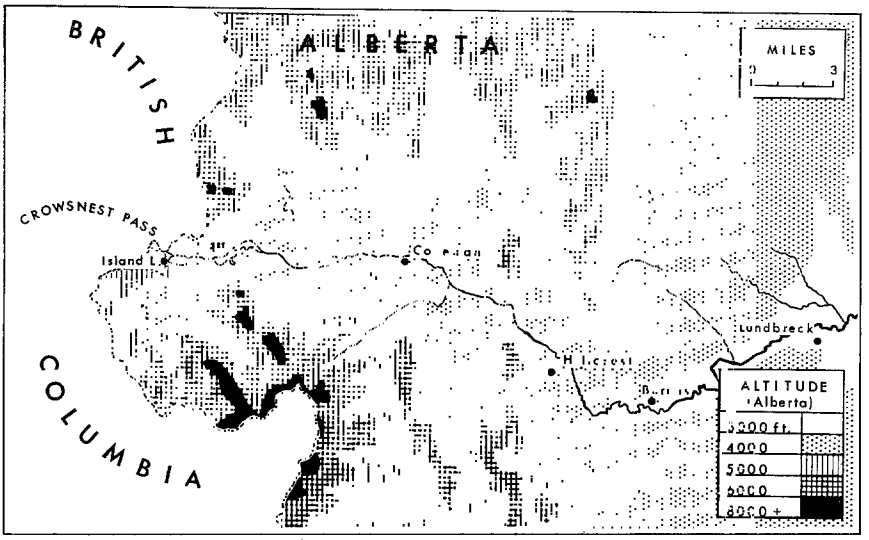


Fig. 2. The Crowsnest River valley area where *Chilocorus* hybrid populations were sampled. Altitude are depicted only in Alberta, its border with British Columbia being the height-of-land

Mainly for technical reasons and economy of time, observation of meiosis has been confined to males, females being classified only by the morphology and number of oogonial metaphase chromosomes in individuals that had been fed colchicine in honey (details in SMITH, 1965). In Canada, *Chilocorus* species overwinter as adults. Such overwintered and otherwise old, late-season males rarely contain primary spermatocytes at metaphase. They, also, were therefore pretreated with colchicine, which then permitted classification only through gonial karyotype analysis, rather than providing the more informative data to be derived from first metaphase pairing relationships of homologues. Slides were prepared as squashes, after fixation in modified Kahle's fluid, and stained in basic fuchsin-methyl green. Results are summarized in Tables 1 and 2. All photographs were taken at an original magnification of *ca* × 1,900 and are reproduced at the magnification stated in the legends.

Observations

1. C. tricyclus

Since 1957, samples of *tricyclus* have been examined from 26 different localities in the interior of British Columbia and four to the immediate south in the State of Washington (Fig. 1). With the exception of two of

the 1964 samples, which included chromosomally mutant heterozygotes (SMITH unpub.), no deviating karyotypes have ever been encountered. The chromosome number of *tricyclus* is 2n = 20, giving a meiotic formula of 3 ring II + 6 non-ring, or "rod", II + 1 neo-XY (♂):XX (♀) II (Fig. 3, a ♂ from Grand Forks, B.C.). As detailed elsewhere (SMITH, 1965) all chromosomes other than the euchromatic acrocentric Y are metacentric. The X chromosomes and the six ring-forming autosomes have both arms euchromatic—they are "monophasic"; the 12 rod-forming autosomes have one arm euchromatic and the other heterochromatic—they are "diphasic". It is because chiasmata are without exception restricted to euchromatic arms and invariably limited to one per arm-pair that monophasics form ring bivalents and diphasics form rod bivalents.

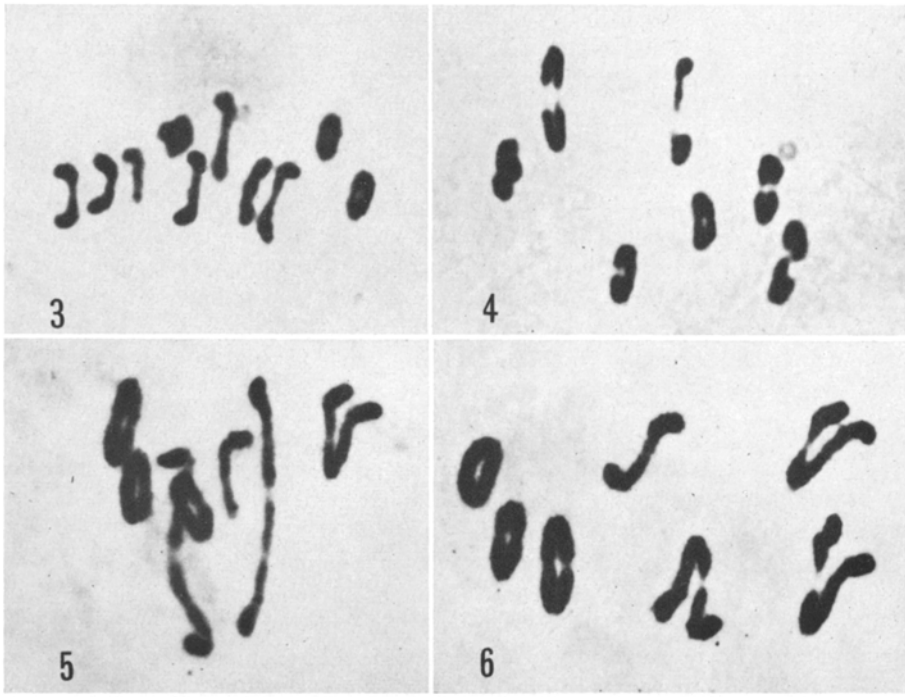
2. *C. hexacyclus*

This species has been studied from the only four general areas in which it is known to occur (Fig. 1). It has a diploid number of 14 chromosomes and a meiotic formula of 6 ring II + 1 neo-XY (♂):XX (♀) II (Fig. 4, a ♂ from Conquest, Sask.). The two arms of each of the 12 autosomes and the X are euchromatic and hence these chromosomes are monophasics. Furthermore, the Y chromosome in this species is metacentric and diphasic, not acrocentric and euchromatic as it is in *tricyclus*; consequently it also forms a rod bivalent.

3. F_1 *hybrids*

To aid comprehension of the descriptions and analyses of first metaphase associations in the various natural hybrid males, Figs. 5 and 6 are inserted. These illustrate the interspecific chromosome homologies as established in laboratory hybrids between *C. tricyclus* from Okanagan Centre, B.C., and *C. hexacyclus* from Conquest, Sask. (SMITH, 1959). Note that in these reciprocal F_1 males (1) the three monophasic ring bivalents of *tricyclus* find identical partners in *hexacyclus*, (2) the six diphasic rod bivalents of *tricyclus* are homoeologous in pairs with the remaining three ring bivalents in *hexacyclus* and thus form three associations of three chromosomes, or trivalents for short and (3) the X and Y chromosomes of the two species are likewise homoeologous but differ morphologically by the Y being acrocentric when *tricyclus* (Fig. 5) and metacentric when *hexacyclus* (Fig. 6) is the male parent. Pairing is near perfect in hybrid males, which therefore form close to the maximum of 13 chiasmata per nucleus, but the centromeres of the trivalents are often linearly orientated in a nondisjunctional arrangement (Fig. 5) rather than convergently (Fig. 6).

Since monophasic and diphasic chromosomes react differently to colchicine, the autosomes that are ring-forming in *hexacyclus* but

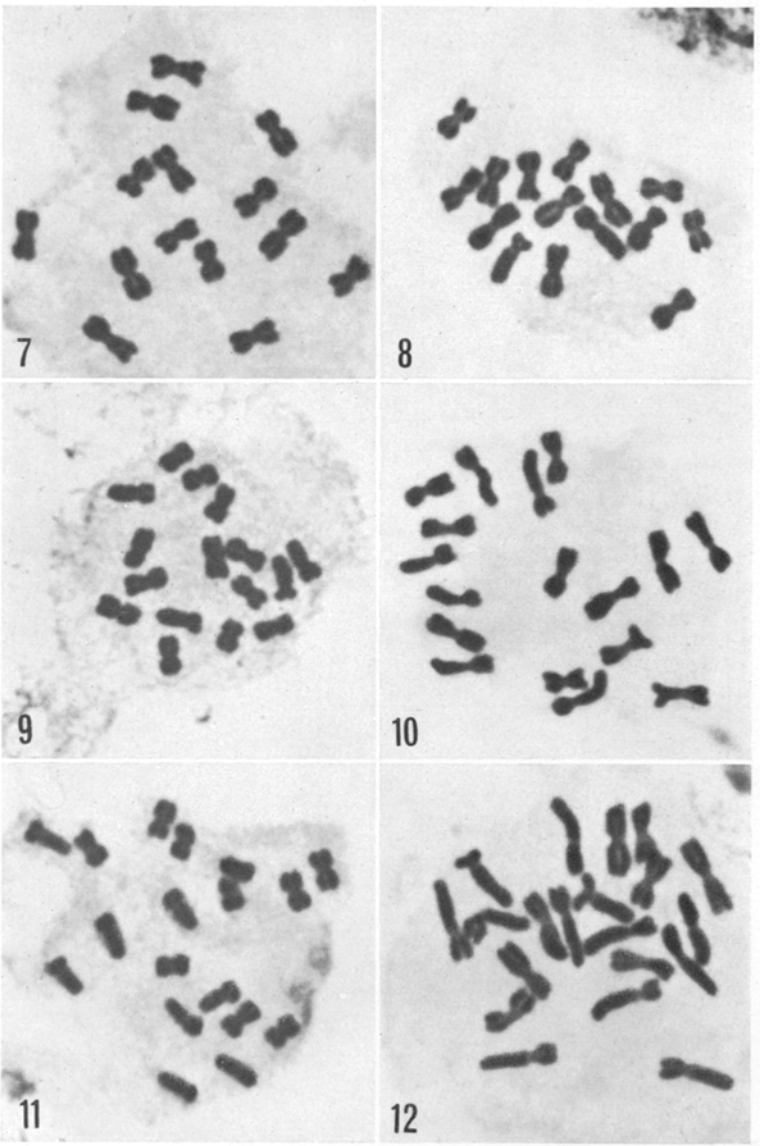


Figs. 3—6. Primary spermatocyte metaphases in side view (*ca* x 1,900). Fig. 3. *C. tricyclus* from Grand Forks, B.C.; 3 ring II + 6 rod II + XY—acrocentric II. Fig. 4. *C. hexacyclus* from Conquest, Sask.; 6 ring II + XY-metacentric II. Fig. 5. *C. hexacyclus* ♀ x *C. tricyclus* ♂; 3 ring II + 1 convergent III + 2 linear III + XY-acrocentric II. Fig. 6. *C. tricyclus* ♀ × *C. hexacyclus* ♂; 3 ring II + 3 convergent III + XY-metacentric II

rodforming in *tricyclus* can be distinguished in gonial mitoses after, but only after, pretreatment. In this way, because a monophasic is the equivalent of two diphasics, chromosome counts are verifiable.

4. The Crowsnest River Valley Community

a) Constitution by chromosome number. 1957: The first collection received from the Crowsnest Pass area came from Coleman (Fig. 2) and consisted solely of *hexacyclus*, but it was too small to state categorically that other entities were absent from the community that year. The material was used for cytogenetic studies conducted in California during 1957—1958 (SMITH, 1959, 1960). 1961: No further samples were received from the area until 1961, after studies of chromosomal polymorphism in *C. stigma* had been resumed (SMITH, 1962). The collection, made at nearby Hillcrest (Fig. 2), being a routine survey sample, i.e., not a "special" collection, totalled only six specimens, but they nevertheless proved sufficient to launch the present investigation. Three of the six had 14 (Fig. 13, a ♂), one had 20 (Fig. 12, a ♀) and one each had 15 (Fig. 8, a ♀)



Figs. 7—12. Colchicine-treated oogonial metaphase plates (*ca* × 1,900). Fig. 7. 2n = 14; all chromosomes monophasic. Fig. 8. 2n = 15; 13 monophasics + 2 diphasics. Fig. 9. 2n = 16; 12 monophasics + 4 diphasics. Fig. 10. 2n = 17; 11 monophasics + 6 diphasics. Fig. 11. 2n = 18; 10 monophasics + 8 diphasics. Fig. 12. 2n = 20; 8 monophasics + 12 diphasics

and 16 chromosomes. Thus they established the coexistence at Hillcrest of *hexacyclus*, *tricyclus* and hybrid-derivative chromosome numbers. Moreover, one of the 14-chromosome males carried a typical, acrocentric, *tricyclus*-type Y chromosome (Fig. 14 and Table 1).

Table 1. *Frequencies of Chilocorus Chromosome Numbers in the Crowsnest River Valley by Year, Locality and Type of Y Chromosome, and Percent Hexacyclus Y chromosomes*

Year	Locality	Sex	Number of individuals with 2n =							Y chr.	Total	% *hexa* Y
			14	15	16	17	18	19	20			
1961	Hillcrest	♂	2							*hexa*	2	50
		♂	1		1					*tri*	2	
		♀		1					1		2	
1962	Coleman	♂	14				1			*hexa*	15	100
		♂								*tri*	0	
		♀	8			1					9	
	Hillcrest	♂	3		1					*hexa*	4	80
		♂				1				*tri*	1	
		♀	6			4			1		11	
	Lundbreck	♂	1							*hexa*	1	50
		♂							1	*tri*	1	
		♀	3		1	3					7	
1963	Coleman	♂	21	2	1	4				*hexa*	28	74
		♂	2			6		1	1	*tri*	10	
		♀	16	1		4			1		22	
	Hillcrest	♂	11	6	1	2	1			*hexa*	21	66
		♂	2			7			2	*tri*	11	
		♀	13	3	1	15	2		1		35	
1964	Island L.	♂								*hexa*	0	0
		♂				1			1	*tri*	2	
		♀	2	1							3	
	Coleman	♂	65	12	7	6				*hexa*	90	71
		♂	16	6	1	9	2	2	1	*tri*	37	
		♀	19	1	4	1	2				27	
	Burmis	♂	14	1	4	4				*hexa*	23	64
		♂	3	1	1	5		1	2	*tri*	13	
		♀	2	3	3	1					9	
	Lundbreck	♂	1							*hexa*	1	50
		♂				1				*tri*	1	
		♀	1								1	
Total		♂	132	21	14	16	2			*hexa*	185	70
		♂	24	7	3	30	2	4	8	*tri*	78	
		♀	70	10	9	29	4		4		126	
Grand total			226	38	26	75	8	4	12		389	

1962—3: Larger, especially requested collections during the following two years netted 176 individuals with satisfactory cytology (Table 1). Among them were 100 with 14 (Fig. 7, a ♀) and seven with 20 chromosomes (Fig. 21, a ♂), the balance of 69 covering all chromosome numbers

from 15 to 19 (Figs. 15—20, ♂♂ and 9—11, ♀♀) and thus including the expected F_1 hybrid number 17. Of the 20 males in this last category, six carried the diagnostic *hexacyclus*-type, metacentric Y (Fig. 17) and 14 the diagnostic *tricyclus*-type, acrocentric Y (Fig. 18). The 27 females with 17 chromosomes, due to their lack of morphologically different X chromosomes, could not be similarly subdivided. As in 1961, two types of 14-chromosome males occurred: 50 with a metacentric Y (Fig. 13) and four with an acrocentric (Fig. 14). The four males among the seven individuals with 20 chromosomes all possessed an acrocentric Y (Fig. 21 and Table 1).

1964: A total of 207 beetles, the largest one-year sample analysed, provided satisfactory preparations (Table 1). As in 1962—63, all numerical classes were represented but for the first time both types of Y chromosome occurred in each of the 14- to 17-chromosome categories, although metacentric Ys were again absent from those with 19 and 20, and also in 1964 from those with 18 chromosomes.

Altogether, over the years 1961 to 1964, 389 individuals were classified cytologically, of which 263 were males. The numbers in each numerical category are given in Table 1. In hybrids six of the gonial autosomes cannot be distinguished as to parental origin; they are the six ring-forming monophasics common to *hexacyclus* and *tricyclus* (Figs. 5 and 6). But if the segmental changes resulting from prior crossing-over are ignored any monophasics in excess of six and their corresponding diphasics can be designated as to parental origin, as can the Y also in males. Accordingly 50 per cent of the total basic complement in males and, their two X chromosomes being superficially similar, 50 per cent of the basic autosomal complement in females can be defined. However, unless the meiotic pairing relationships are determinable (Table 2), which is not feasible for females, only four of the seven numerical classes can be specified as to the relative homologies of monophasic and diphasic autosomes and, then, only after pretreatment with colchicine.

Table 2. *Frequency of Male Chilocorus Meiotic Karyotypes in the Crowsnest River Valley: 1961—1964*

2n =	14	15	16		17		18		19	20	Total
Class	1	3	5a	5b	6a	6b	7a	7b	4	2	
Karyotypes*	6⊙	5⊙ 1V	5⊙ 2R	4⊙ 2V	4⊙ 1V 2R	3⊙ 3V	4⊙ 4R	3⊙ 2V 2R	3⊙ 1V 4R	3⊙ 6R	
$+XY^m$	104	19	0	12	1	11	0	1	0	0	148
$+XY^a$	19	5	0	2	0	23	0	2	2	6	59

* ⊙ = ring bivalent; V = trivalent; R = unichiasmate rod bivalent; superscript m and a respectively = meta- and acrocentric Y chromosome.

These four classes are:

(1) 2n = 14—all autosomes monophasic (Figs. 7, 13 and 14);

(2) 2n = 20—six autosomes monophasic and 12 diphasic (Figs. 12 and 21);

(3) 2n = 15—one monophasic replaced by two diphasics, i.e., a single structural-numerical heterozygote (Figs. 8 and 15);

(4) 2n = 19—two diphasics replaced by one monophasic, likewise a single structural-numerical heterozygote (Fig. 20).

The three dual classes (Table 2) are:

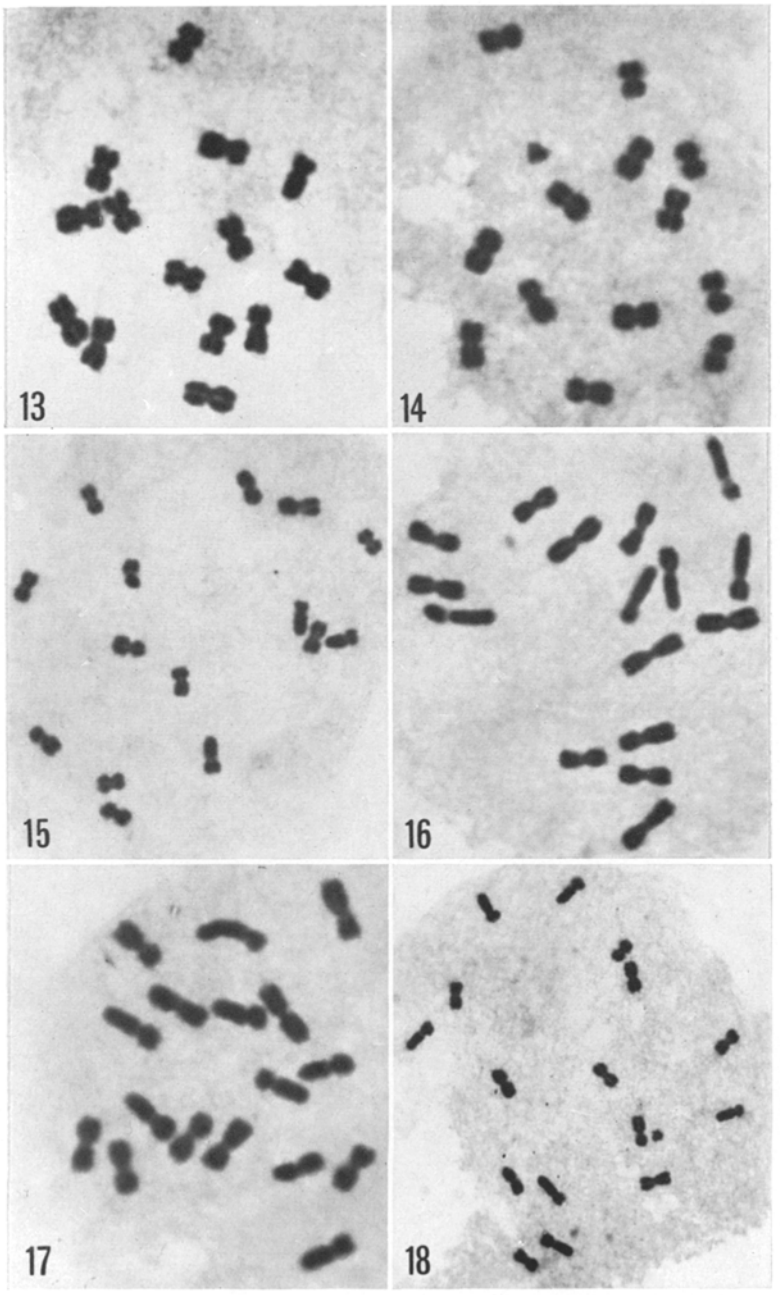
(5) 2n = 16, one with a pair of homologous monophasics replaced by two pairs of twin-diphasics—a "true-breeding" structural-numerical homozygote or neo-diploid (subclass 5a) and one with two nonhomologous monophasics replaced by their respective twin-diphasics—a double structural-numerical heterozygote (subclass 5 b);

(6) 2n = 17, one with a pair of homologous monophasics replaced by two pairs of twin-diphasics plus a third monophasic replaced by its twin-diphasics—a single structural-numerical heterozygote (subclass 6 a) and one with three nonhomologous monophasics replaced by their respective twin-diphasics—a triple structural-numerical heterozygote (subclass 6 b);

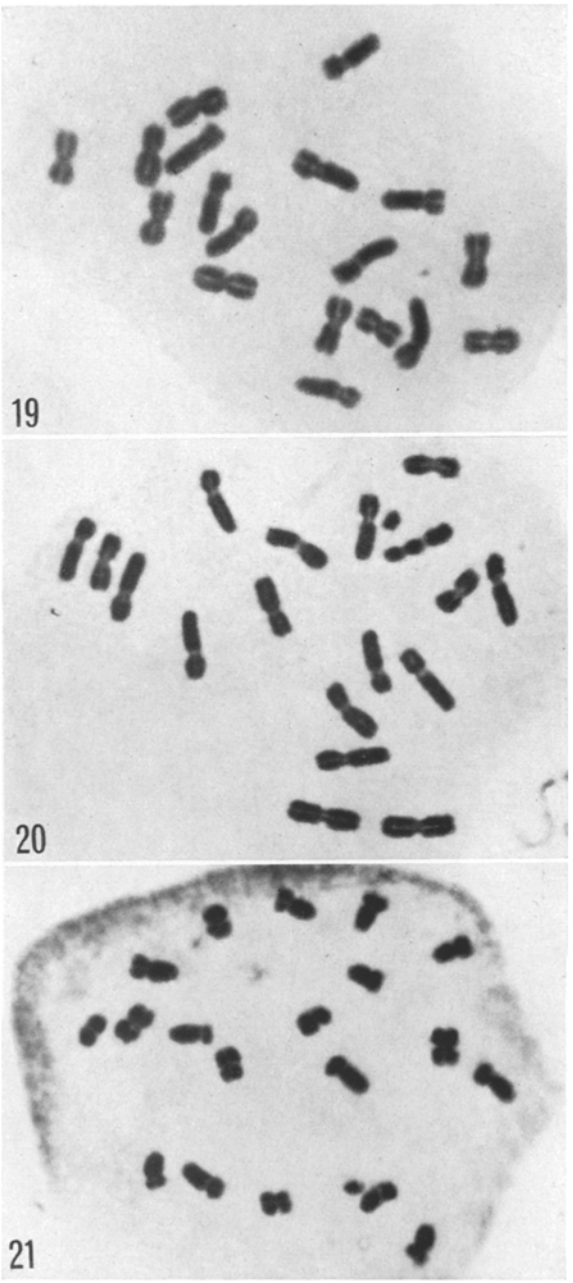
(7) 2n = 18, one with two pairs of homologous monophasics replaced by four pairs of twin-diphasics—a "true-breeding" structural-numerical homozygote or neo-diploid (subclass 7a) and one with one pair of homologous monophasics replaced by two pairs of twin-diphasics plus two nonhomologous monophasics replaced by their respective twin-diphasics —a double structural-numerical heterozygote (subclass 7b).

Finally, in all classes males but not females can be further categorized on the basis of their type of Y chromosome.

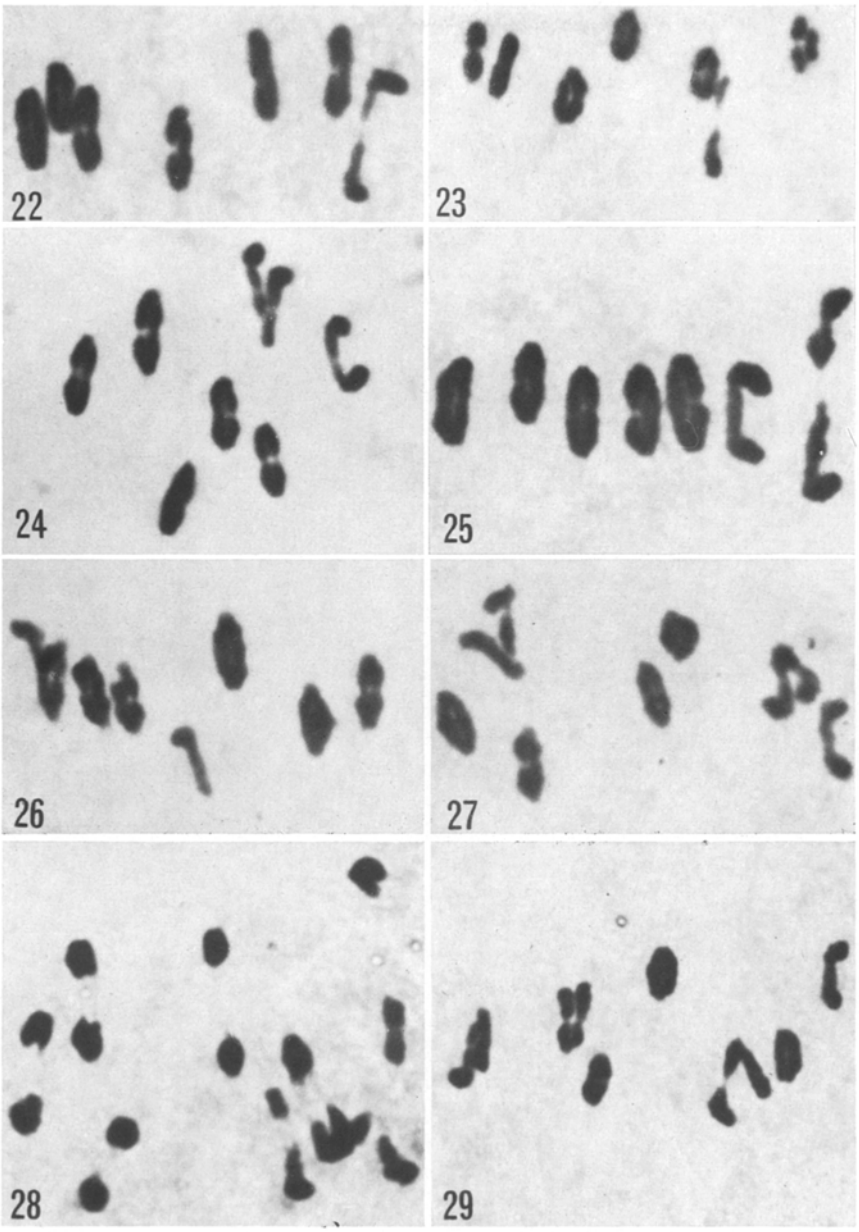
b) Male meiotic chromosome associations. Among the 156 males with 2n = 14 chromosomes, 132 carried a metacentric Y and 24 an acrocentric Y. Of these males, 104 (Fig. 22) and 19 (Fig. 23) respectively contained first metaphases (Table 2); the former are therefore either pure *hexacyclus* or indistinguishable neo-diploids whereas the latter are all neo-diploids. Among the several hundred cells examined in the two types, pairing and co-orientation of the centromeres were perfectly regular, both between the sex chromosomes and between the autosomes. The latter almost invariably formed rings, a ring only rarely being replaced by a rod and never by two univalents. Thus the chiasma frequency per cell approached closely or usually equalled the maximum of 13.0 possible. Clearly despite some 20 % of the rings in the Crowsnest River valley being primary and others secondary (crossover) recombinations of *hexacyclus* and *tricyclus* homoeologues (Table 6), there is no obvious chromosome barrier to full fertility of 14-chromosome individuals irrespective of their "genotypic"



Figs. 13—18. Colchicine-treated spermatogonial metaphase plates (*ca* × 1,900 except Figs. 15 × 1,400 and 18 × 1,100). Fig. 13. 2n = 14; 13 monophasics + diphasic *hexa*-type Y. Fig. 14. 2n = 14; 13 monophasics + minute acrocentric *tri*-type Y. Fig. 15. 2n = 15; 12 monophasics + 2 diphasics + *hexa*-type Y. Fig. 16. 2n = 16; 11 monophasics + 4 diphasics + *hexa*-type Y. Fig. 17. 2n = 17; 10 monophasics + 6 diphasics + *hexa*-type Y. Fig. 18. 2n = 17; 10 monophasics + 6 diphasics + *tri*-type Y



Figs. 19—21. Colchicine-treated metaphase plates (*ca* × 1,900). Fig. 19. 2n = 18; 9 monophasics + 8 diphasics + *hexa*-type Y. Fig. 20. 2n = 19; 8 monophasics + 10 diphasics + *tri*-type Y. Fig. 21. 2n = 20; 7 monophasics + 12 diphasics + *tri*-type Y



Figs. 22—29. Primary spermatocyte metaphases and a meta-anaphase in side view (*ca* × 1,900). Fig. 22. 2n = 14 *C. hexacyclus* or neo-diploid; 6 ring II + XY-metacentric II. Fig. 23. 2n = 14 neo-diploid; 6 ring II + XY-acrocentric II. Fig. 24. 2n = 15 single heterozygote; 5 ring II + 1 convergent III + XY-metacentric II. Fig. 25. 2n = 15 single heterozygote; 5 ring II + 1 linear III + XY-metacentric II. Fig. 26. 2n = 15 single heterozygote; 5 ring II + 1 convergent III + XY-acrocentric. Fig. 27. 2n = 16 double heterozygote; 4 ring II + 2 convergent III + XY-metacentric II. Fig. 28. 2n = 16 double heterozygote; meta-anaphase showing 4 ring II disjoining or disjoined + 1 convergent III (second from right) + 1 linear III (right) + XY-acrocentric II. Fig. 29. 2n = 17 triple heterozygote; 3 ring II + 3 convergent III + XY-metacentric II

constitution. This is also demonstrated by the perfection of first anaphase disjunction.

Twenty-eight males had 15 chromosomes, 21 with a metacentric and seven with an acrocentric Y; 19 (Figs. 24 and 25) and five (Fig. 26) respectively provided first metaphases (Table 2). In about two-thirds of the cells the single trivalent displayed a convergent, or disjunctional, orientation (Figs. 24 and 26), with the bivalents as in essentially all other numerical classes pairing and disjoining regularly to form euploid[1] sperms. In the remaining one-third of the cells the trivalent was linearly orientated (Fig. 25) so as to give rise to equal numbers of aneuploid duplication (one monophasic plus one diphasic) and aneuploid deficiency (only one instead of two diphasics) sperms. These will provide viable recombinants only when fertilizing the complementary types of eggs (*v.* later).

Of the 17 males with 16 chromosomes, 14 carried a metacentric and three an acrocentric Y; 12 (Fig. 27) and two (Fig. 28) respectively had first metaphases (Table 2). Although, as mentioned earlier, the four diphasics in this numerical category may be of two constitutionally different types, all 14 males with first metaphases belonged in subclass 5 b, the double heterozygote with four ring bivalents, two trivalents and the XY bivalent (Table 2); thus neo-diploids of subclass 5 a were not observed. In primary spermatocyte metaphases 55 % of the cells showed malorientation of one (Fig. 28) or both trivalents (Table 3), thereby assuring a higher potential sterility than is to be expected of 15-chromosome heterozygotes.

Forty-six males had the F_1 number of 17 chromosomes, 16 with a metacentric and 30 with an acrocentric Y; 12 (Figs. 29 and 30) and 23 (Fig. 31) respectively provided first metaphases (Table 2). In all but one of these the six diphasics were associated in pairs with a monophasic to form three trivalents, i.e., they were subclass 6 b males. In the 35th male, which had a metacentric Y, in addition to four ring bivalents and the XY, two of the six diphasics were paired with a monophasic to form a trivalent, the remaining four being present as rod bivalents (Fig. 32), i.e., it was the sole representative of subclass 6 a. Averaged over three triple heterozygotes, in only about one-quarter of the cells were all three trivalents orientated disjunctionally (Fig. 29 and Table 3). Depending on how many trivalents are orientated linearly, these cells will give rise to single, double and triple duplication-deficiency sperms (Table 8 B). The lone subclass 6 a male had the ability to produce euploid sperms equal to that of the single heterozygotes with 15 chromosomes that have already been described.

[1] The term "euploid" is used herein for gametes that carry the full haploid complement of 12 euchromatic arms vs. aneuploid gametes that carry more or fewer than 12 such arms.

Table 3. *Relative Trivalent Orientation at First Meiotic Metaphase in Single, Double and Triple Chilocorus Heterozygotes in the Crowsnest River Valley*

Autosomal karyotype *	No. of cells	No. of cells with 1, 2 or 3 V orientated				% trivalents		% cells orientated
		3V	2V	1V	0V	Orient.	Malor.	
3⊙+1V+4R	42	—	—	28	14	.67	.33	.67
5⊙+1V	65	—	—	49	16	.75	.25	.75
5⊙+1V	77	—	—	52	25	.68	.32	.68
5⊙+1V	116	—	—	76	40	.66	.34	.66
3⊙+2V+2R	44	—	14	17	13	.50	.50	.32
3⊙+2V+2R	100	—	28	47	25	.48	.52	.28
4⊙+2V	100	—	45	42	13	.66	.34	.45
3⊙+3V	100	13	36	38	13	.50	.50	.13
3⊙+3V	100	17	41	30	12	.54	.46	.17
3⊙+3V	100	47	31	21	1	.75	.25	.47
1V	300	—	—	205	95	.68	.32	.68
2V	244	—	87	106	51	.57	.43	.36
3V	300	77	108	89	26	.60	.40	.26

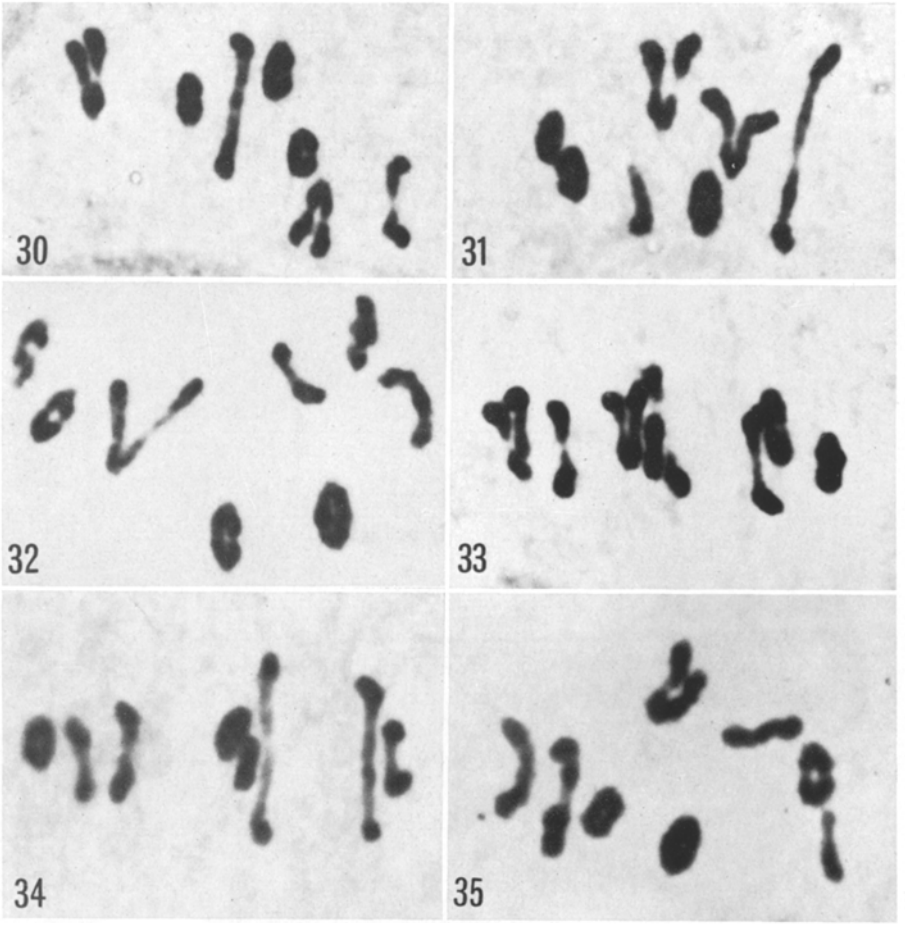
* ⊙ = ring bivalent; V = trivalent; R = unichiasmate rod bivalent.

Four males had 18 chromosomes, two with a metacentric and two with an acrocentric Y; one (Figs. 33 and 34) and two (Fig. 35) respectively were studied at first metaphase (Table 2). Allthree, in having three rings, two trivalents, two rods and an XY pair, belonged in subclass 6b. The four ring + four rod + XY subclass 6a neo-diploid failed to materialize. Malorientation of the two trivalents in the two individuals studied in detail was higher than in the 16-chromosome male, i.e., *ca.* 70% of the cells had one (Fig. 35) or both (Fig. 34) trivalents linearly orientated (Table 3).

Four males had 19 chromosomes, all with an acrocentric Y. Two contained first metaphases (Fig. 36 and Table 2), wherein the single trivalent showed 33% malorientation (Table 3), a figure agreeing with the average calculated from three 15-chromosome heterozygotes.

Finally, all eight males with 20 chromosomes had an acrocentric Y. The six with primary spermatocyte metaphases (Table 2) were indistinguishable in meiotic regularity from pure *tricyclus* (Fig. 3), which in fact they might well be.

c) Segregational sterility. As stated earlier, centromere co-orientation in bivalents was invariably disjunctional and chiasma formation between monophasic and diphasic autosomal homoeologues, as also between the X and Y chromosomes, was essentially perfect. For example, among the 844 cells, containing 3604 autosomal bivalents, 844 pairs of sex chromosomes and 1688 trivalents, that are recorded in Table 3, only one instance of asynapsis was observed (between sex chromosomes)—a negli-



Figs. 30—36. Primary spermatocyte metaphases in side view (*ca* × 1900). Fig. 30. 2n = 17 triple heterozygote; 3 ring II + 2 convergent III + 1 linear III + XY-metacentric II. Fig. 31. 2n = 17 triple heterozygote; 3 ring II + 2 convergent III + 1 linear III + XY-acrocentric II. Fig. 32. 2n = 17 single heterozygote; 4 ring II + 2 rod II + 1 convergent III + XY-metacentric II. Fig. 33. 2n = 18 double heterozygote; 3 ring II + 2 rod II + 2 convergent III + XY-metacentric II. Fig. 34. 2n = 18 double heterozygote; 3 ring II + 2 rod II + 2 linear III + XY-metacentric II. Fig. 35. 2n = 18 double heterozygote; 3 ring II + 2 rod II + 1 convergent III + 1 linear III + XY-acrocentric II. Fig. 36. 2n = 19 single heterozygote; 3 ring II + 4 rod II + 1 convergent III + XY-acrocentric II

gible amount. All sterility, which cannot of course be directly measured in these field samples, is therefore attributed to malorientation of trivalents. The data in Table 5 are so analyzed. (Incidentally, throughout the study no chromosomally unbalanced individual was encountered.)

In accordance with MENDEL's principle of segregation, the relative orientation of convergent trivalents in triple, double and single heterozygotes, both among themselves and with respect to the X acrocentric-Y

Table 4. *Relative First Metaphase Orientation of the tricyclus Y Chromosome and the Trivalents in Triple, Double and Single Chilocorus Heterozygotes in the Crowsnest River Valley*

Heterozygote	⋀⋀⋀ L	⋀⋀⋀ ⅂	⋀⋀⋁ L	⋀⋀⋁ ⅂	⋀⋀ L	⋀⋀ ⅂	⋀⋁ L	⋀ L	⋀ ⅂
Triple	6	14	30	26	25	21	63	37	52
Double	—	—	—	—	25	23	53	43	36
Single	—	—	—	—	—	—	—	25	24
Total Obs.	6	14	30	26	50	44	116	105	112
Ratio exp.	1 :	1 :	3 :	3	1	: 1 :	2	1 :	1

bivalent, was close to random (Table 4), the deviation in the smalles class being ascribable to sampling error. Consequently, the standard mendelian chequer-board technique has been applied in calculating recombination frequencies (Tables 5, 8 and 10).

Despite the difficulty introduced by inter-slide variability in determining precise values for the relative frequencies of convergent vs. linear trivalents and hence for orientated (p) vs. malorientated (q) metaphases, when they are estimated from the means for single, double and triple heterozygotes, it is possible for methodological purposes to compare sterility in backcrosses with that in *inter se* matings (Table 5).

In 15- and 19-chromosome single heterozygotes, with *ca* 68 % of the trivalents convergent (Table 3) and hence *ca* .68 of the metaphases orientated, euploid gametes with seven and eight chromosomes will be formed in equal numbers. Should one mate *inter se*, i.e., with an individual having a homologous trivalent (the chance of which is only $^1/_3$ since there are three different trivalents in the population), these euploid gametes will recombine in the ratio of one 14-chromosome homozygote : two 15-chromosome heterozygotes : one 16-chromosome homozygote. If, however, the mating had not been *inter se*, the 16-chromosome class would consist solely of double heterozygotes.

The 32 % aneuploid gametes are of four types, as follows: If we designate the *hexacyclus* monophasic in the trivalent as "*A*" and its arms as "*a*" and "*b*", each will pair with the corresponding *tricyclus* diphasic *a* or *b* chromosome. Following a linear alignment at metaphase, the *A* can accompany either *a* or *b* to the pole. The four resulting aneuploid gametes are *Aa* and *Ab* (duplication types) and *a* and *b* (deficiency types). Of the 16 possible chequer-board recombinants only four will be *aAb* euploids and consequently all four will be single heterozygotes, the remainder being inviable aneuploid zygotes determining 75 % sterility. This of course holds only for *inter se* matings, for if single heterozygotes are mated at random, with three different trivalents ("*aAb*, *cCd* and *eEf*") capable of forming 12 different types of aneuploid gametes, the chance of complementary aneuploid gametes uniting is reduced to one-twelfth (Tables 5 and 9).

Table 5. *Relative Sterilizing Effect of Trivalent Malorientation in Backcross vs. Inter se Single, Double and Triple Chilocorus Heterozygotes in the Crowsnest River Valley*

Hetero-zygote	p	q_1	q_2	q_3	Viable recombinations		% an-euploid contrib. to fertility	% sterility	
					ex euploids	ex aneuploids*		*inter se*	back-cross
Single	.68	.32			$p^2 = .4624$	$^1/_4 q_1^2 = .0256$	5.25	51.2	32.0
Double**	.36	.44	.20		$p^2 = .1296$	$^1/_8 q_1^2 + ^1/_{16} q_2^2 = .0267$	17.09	84.37	64.0
Triple	.26	.36	.30	.09	$p^2 = .0676$	$^1/_{12} q_1^2 + ^1/_{48} q_2^2 + ^1/_{64} q_3^2$ $= .0128$	15.92	91.96	74.0

* In all fractions the denominator is equal to the number of different types of aneuploid gametes formed. For crosses other than *inter se*, the denominator is three times as large except in triple heterozygotes which have any or all three trivalents subject to malorientation.

** More precise values of q_1 and q_2 are .434 and .21 but .44 and .20 have been substituted to simplify calculations.

To return to *inter se* matings; altogether, 14-, 15- and 16-chromosome viable progeny are expected in a ratio of $^1/_4\ p^2 : ^1/_2\ p^2 + ^1/_4\ q^2 : ^1/_4\ p^2$; in other words an excess of heterozygotes over homozygotes should always materialize from such strictly *inter se* matings (Tables 5 and 8 A and B). Accordingly, inviability among zygotes from such matings is expected to be just over one-half, in contrast to an estimated one-third following backcrossing to the parental species or to neo-diploids (Table 5).

Inter se matings of double heterozygotes with 16 (or 18) chromosomes segregate progeny with 14 to 18 (or 16 to 20) chromosomes. Because the two trivalents orientate independently (Table 4), q consists of two discrete fractions: q_1 for cells with only one trivalent linear and q_2 for those

with two. Estimated from the formula given in Table 5, only *ca* 16 % of the recombinants are expected to be viable and of these the $q_1^2+q_2^2$ component formed by complementary aneuploid unions (*ca* 3 %) will all be double heterozygotes (Table 9). Similarly in triple heterozygote matings (all of which are *inter se*) calculated fertility is reduced to *ca* eight per cent of which about one-sixth results from complementary aneuploid unions that may be either single or triple heterozygotes (Table 9). Further consideration of segregation and sterility is deferred to the Discussion.

Discussion

Of the 389 insects studied from the Crowsnest River valley community between 1961 and 1964, 263 were males and only 126 females (Table 1). The excess number of heterogametics—this apparent contravention of HALDANE's rule—is not in reality a true imbalance in the sex ratio; it stems from a calculated priority afforded males during periods of heavy work-load, the choice being made on the basis of the more revealing information that can be extracted from a study of males. This is in fact attested by the deficiency of females being equally large in the 14- and 20-chromosome categories (Table 1), the two that should be least influenced by any possible sex chromosomal-autosomal-cytoplasmic interaction.

A somewhat more puzzling departure from an expected 1:1 ratio lies in the frequencies of 17-chromosome males with metacentric (16) vs. acrocentric (30) Y chromosomes (Table 1). This can hardly be written off as due to sampling errors, for in all seven year-locality samples that included 17-chromosome males acrocentric-Y males proved the more common. Although an interpretation based on reciprocal differences in Y-chromosome "compatibility" is remotely conceivable, I am even less inclined to the alternative view that the underlying cause is a sex differential in sexual isolation between *hexacyclus* and *tricyclus*, with females of the former yielding more readily to hybridization, despite this being a by no means novel phenomenon. This latter possibility is considered because a limited number of laboratory pairings of the two species in 1957—58 resulted in fewer failures when *tricyclus* was used as the male parent than in the reciprocal (SMITH unpub.). It seems that since *tricyclus* can enter the area of overlap only as adults, the logical explanation is that most invading females had already mated and at first at least produced only *tricyclus* progeny (these species are polygamous), whereas the accompanying males were from the start free to mate at random. Of course with *hexacyclus* and *tricyclus* X chromosomes indistinguishable, the comparable excess expected among females cannot be demonstrated.

The major role played by a preponderance of 17-chromosome males having an acrocentric Y is to distort the estimate of *tricyclus* replacement

chromosomes when calculated from Y chromosomes alone (29.7 %) as against that when monophasic and diphasic autosomes are included (19.7 %, Table 6). Since the Y values are thereby reduced to $^1/_7$ (Fig. 37), the latter method of calculation also smooths out the fluctuations in the Y curve. When the percentages of *tricyclus* replacement chromosomes are plotted within chromosome-number categories, the only effect that the absolute number of individuals per category has on the slope of the line is through the Y chromosomes (Fig. 38). Thus the slope for any F_2

Table 6. *Percent Hexacyclus Chromosomes Replaced by Tricyclus Diphasic Autosomes and Y Chromosomes in the Crowsnest River Valley by Sex and Grouped: 1961—1964*

	Categories with 2n =							Percent
	14	15	16	17	18	19	20	
Total no. of *hexa* Y	132	21	14	16	2	0	0	
Total no. of *tri* Y	24	7	3	30	2	4	8	
% *tri* Y within categories	15.4	25.0	17.6	65.2	50.0	100.0	100.0	29.7
% *hexa* chrs replaced: ♂♂	2.2	17.9	31.1	52.2	64.3	85.7	100.0	19.7
Total no. of females	70	10	9	29	4	0	4	
% *hexa* chrs replaced: ♀♀	0.0	16.7	33.3	50.0	66.7	(83.3)	100.0	20.5
% *hexa* chrs replaced: ♂♂ + ♀♀	1.6	17.6	31.8	51.4	65.4	85.7	100.0	19.9
% replacement by category	0.9	1.7	2.1	9.8	1.3	0.9	3.1	19.9

progeny is precisely the same as that for the combined backcross progenies (Fig. 38). The line for the F_2 is however affected by the relative numbers of Y^h and Y^t in the parental F_1 population for they determine the point of origin of the line (Fig. 37); their influence is nevertheless depressed by the inclusion of females in the analysis. When the 19.9 % replacement chromosomes in the grouped four-year sample are assigned proportionately to the seven chromosome-number categories (Table 6, bottom line), about half of them fall in the 17-chromosome class, thus indicating that most of its members are newly arisen first-generation hybrids.

WHITE, CARSON and CHENEY (1964), investigating inter-racial hybrids of the Australian eumastacid grasshopper *Moraba viatica* ERICHSON (2n ♂ = 17 and 19) collected from an area of overlap, found that a relatively high percentage of aneuploid sperms are produced through asynapsis (*ca* 9 %) or malorientation (*ca* 12 %) of the trivalent. These values differ strikingly from those observed herein, where asynapsis is essentially absent but malorientation in single heterozygotes, for example, is almost three times as frequent (Table 3). One of the chromosomes in the *M. viatica* trivalent and the arm with which it is homologous appear to

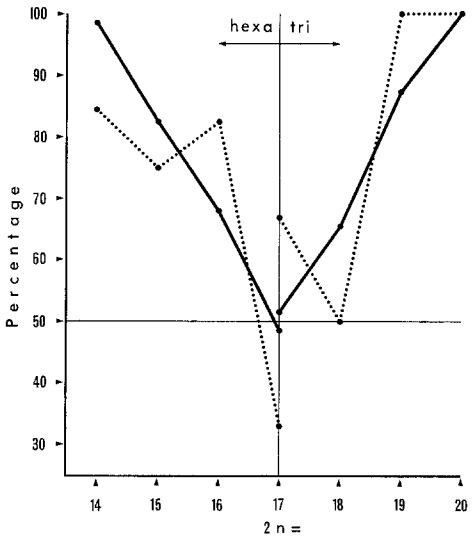


Fig. 37. Observed percent *hexacyclus* chromosomes (left) and *tricyclus* replacement chromosomes (right) per chromosome-number category in the Crowsnest River valley, 1961—1964

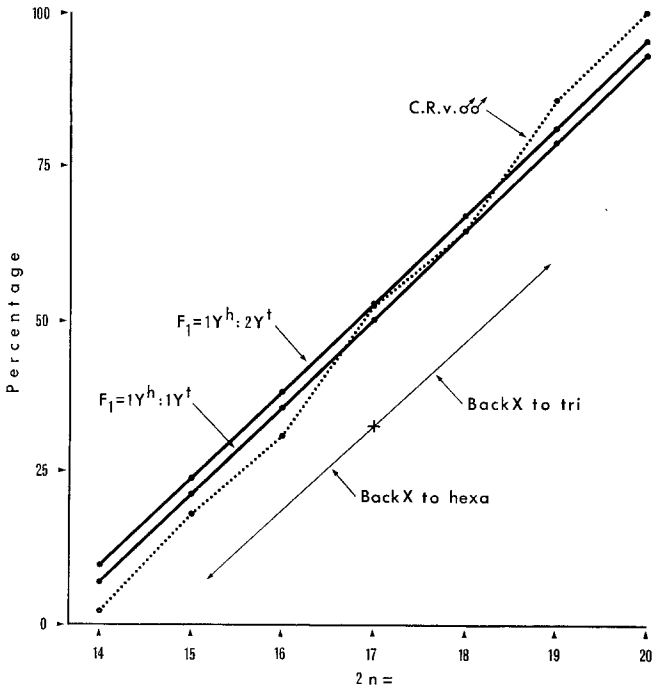


Fig. 38. Expected distribution of percent *tricyclus* replacement chromosomes in F_2s ex F_1 populations having $Y^h:Y^i$ ratios of 1:1 and 1:2 vs. the percentages observed in the Crowsnest River valley, 1961—1964

be only about one-third the length of the third chromosome, and it is this smallest chromosome that occurs as the univalent. The complete synapsis in these *Chilocorus* trivalents is apparently attributable to (1) the equivalent lengths of the chromosome arms involved and (2) the fact that chiasma interference across the centromere is virtually nonexistent.

On the other hand, the considerably higher degree of malorientation in the *Chilocorus* natural hybrids is at first sight more difficult to account for, although it is roughly the same in artificial hybrids (SMITH unpub. and Figs. 5 and 6). In natural numerical heterozygotes in the related chromosomally polymorphic species *C. stigma*, with up to five different fusions including one in the XXXX:XXY sex-determining system, malorientation is negligible (in different individuals only between zero and 0.5 % of the cells contain linear trivalents). It is clear that the centromeres in its trivalents, like those in *M. scurra* RHEN inter-racial hybrids [the "A" and "B" arms of which are likewise of equivalent length (WHITE, 1957)], have adapted to convergent co-orientation. If they had not, the sequential polymorphisms, which occur all across Canada from Nova Scotia to Alberta (SMITH, 1962 and unpub.), would inevitably have eradicated themselves. It is in fact significant that fusion heterozygotes tend to be replaced by fusion homozygotes with progression westwards. It seems perfectly reasonable to conclude that the fusion heterozygosity in *stigma* confers an initial selective advantage (heterosis) that becomes lost with westward progression of the polymorphs (SMITH, 1962). What then remains a paradox is why trivalents in hybrids between *tricyclus* and *hexacyclus*, species that by test-crossing have been shown (SMITH, 1959) to have evolved by centric fusion from an *orbus*-like progenitor with $2n = 22$ chromosomes (homozygous for two fusions), should upon becoming narrowly sympatric have lost their ability to orientate convergently—an ability to which, by analogy with *stigma*'s balanced polymorphisms, they owe their long-term existence as distinct species. Moreover, artificial hybrids between *orbus*, *tricyclus* and *hexacyclus* on the one hand and *stigma* on the other, all of which exhibit high frequencies of univalents, show equally as much malorientation as do those between the first three themselves (SMITH, 1959 and unpub.) and, furthermore, *regardless of the direction in which the cross is made.* Clearly cytoplasmic interaction as the cause is eliminated. It may be that *tricyclus* and *hexacyclus*, unlike the *stigma* polymorphs, arose not via a state of balanced polymorphism but step-wise via "tetracyclus" and "pentacyclus" in a series of marginal populations in which the chromosome mutations quickly became homozygous, so that the partial sterilizing effect of malorientation operated over only a few generations.

It is a matter of general observation (CALLAN and JACOBS, 1957; HUGHES-SCHRADER, 1943; INAMDAR, 1949; JOHN and LEWIS, 1957;

WHITE, 1941) that in orthopteroid insects re-orientation of linear configurations is achieved during the premetaphase stretch, thus at least implying a causal relationship between the two phenomena. But this is most certainly not so in *stigma* heterozygotes where convergent orientation is the rule despite the absence of the prestretch. However, the reverse correlation holds in natural and artificial *Chilocorus* hybrids, for although the prestretch is again absent, so is re-orientation. Thus, for example, in the two testes of a double heterozygote, one with and one without anaphases in the cyst, the former registered 35 and the latter 33 % malorientated trivalents. Comparable values assessed for two single heterozygotes are 32 % with and 34 % without malorientation. Now according to CALLAN and JACOBS (1957) in *Mantis religiosa* (L.), first, lagging univalents arisen by desynapsis block cell division at first metaphase and, second, chromosome bridges resulting from malorientation of trivalents persist at least until second telophase; the two probably automatically preventing formation of aneuploid gametes. In the *Chilocorus* hybrid males under study, univalents are essentially absent and bridge formation totally lacking, and since there is no apparent reason why the situation should differ in females, production of euploid zygotes by recombination of complementary aneuploid gametes is to be expected. It is upon this assumption that the calculations on fertility-sterility are based. Even were centromeres invariably convergent on the female side, male-determined sterility would still be high, equivalent to that calculated for single, double and triple heterozygotes backcrossed to the parental species (Table 5).

Because union of complementary aneuploid gametes can result only in heterozygotes (Table 9), each with segregational sterility equal or close to that of its parents, they in turn play correspondingly minor roles in population-density maintenance. Furthermore, the chance of *inter se* mating, upon which the estimates in Table 5 are based, is remote compared to that of backcrossing to 14-chromosome individuals or outcrossing with other low chromosome-numbered hybrid derivatives (*v.* Table 1 frequencies).

In Table 7 the observed male meiotic autosomal karyotype frequencies are compared with expectation in the F_2 and following backcrossing of the F_1 to *hexacyclus* and to *tricyclus* (*v.* LEWIS and JOHN, 1963, p. 376). As shown in Table 2, among the 207 males classified meiotically the only unmistakable neo-diploids were 13 + acrocentric-Y individuals, no 16- or 18-chromosome homozygotes having been encountered. Now among the F_2 progeny such "pentacyclus" and "tetracyclus" types should each constitute $^3/_{64}$ of the segregants and, being neo-diploids, each should produce only euploid gametes and hence be fully fertile upon inbreeding or upon mating with other chromosomal homozygotes. They cannot,

Table 7. *Theoretical Male Meiotic Autosomal Karyotype Ratios in the F_2 Generation vs. Backcrossing to C. hexacyclus and to C. tricyclus and the Frequencies Observed in the Crowsnest River Valley*

	Triple	Heterozygotes					Homozygotes			
		Double		Single			Parental		Recombinant	
Autosomal karyotypes*	3⊙ 3V	4⊙ 2V	3⊙ 2V 2R	5⊙ 1V	4⊙ 1V 2R	3⊙ 1V 4R	6⊙	3⊙ 6R	5⊙ 2R	4⊙ 4R
Chromosome no.	17	16	18	15	17	19	14	20	16	18
F_2 (exp.)	8	12	12	6	12	6	1	1	3	3
$F_1 \times$ *hexa* (exp.)	1	3	0	3	0	0	1	0	0	0
$F_1 \times$ *tri* (exp.)	1	0	3	0	0	3	0	1	0	0
Observed	34	14	3	24	1	2	123	6	0	0

* ⊙ = ring bivalent; V = trivalent; R = unichiasmate rod bivalent.

however, arise by the F_1 backcrossing to 14- or to 20-chromosome individuals (Table 7). A third karyotype likewise excluded from both backcrosses but not from the F_2 is the 17-chromosome single heterozygote. But although it should materialize in the F_2 50 % more often than 17-chromosome triples, it was in fact encountered only once as against 34 times for the latter (Table 2). On the other hand, 15-chromosome single heterozygotes, with an expectation in the backcross to *hexacyclus* four times that in the F_2, outnumbered 17-chromosome singles 24:1; they moreover outnumbered 19-chromosome singles, from the opposite backcross, 12:1. Obviously the majority of 17-chromosome triple heterozygotes are newly-arisen interspecific hybrids. In summary, a comparison of the expected proportions and observed frequencies establishes that $F_1 \times F_1$ matings must be relatively rare. Moreover, when they do occur ($.19^2$, Table 1) they are, as already stated, detrimental to the extent of their innate segregational sterility (*ca* 92 %, Table 5). Clearly most first-generation hybrids are destined to backcross to *hexacyclus* (*ca* 50 %, Table 1) or outcross with the lower chromosome-numbered hybrid derivatives (*ca* 25 %, Table 1) in proportion to their availability.

The effect of malorientation in the three types of heterozygotes having been estimated (Table 5), the above indication that the Crowsnest River valley community is panmictic can be tested by comparing the observed 1961—1964 frequencies of the different chromosome numbers with expectation based on the Hardy-Weinberg equilibrium formula. The gametic output by such a theoretical population, weighted in accordance with the estimated p and q values (Table 5) and adjusted to include outcrosses (footnote to Table 5), is summarized in Tables 8 A and B and the recombination frequencies are given in Table 10. (This latter table also

Tables 8A and B. *Gametic Output by Grouped Crowsnest River Valley Populations of Chilocorus: 1961—1964*

A. Output of euploid gametes (p values from Table 5)

Chr. no.	Total individuals	p	Proportion of gametes with 2n =			
			7 (a)	8 (b)	9 (c)	10 (d)
14	226	1.00	226.00			
15	38	.68	12.92	12.92		
16	26	.36	2.34	4.68	2.34	
17	75	.26	2.44	7.31	7.31	2.44
18	8	.36		.72	1.44	.72
19	4	.68			1.36	1.36
20	12	1.00				12.00
	389		243.70	25.63	12.45	16.52

B. Output of complementary aneuploid gametes (q values from Table 5)

Chr. no.	Total individuals	q	Proportions of gametes with 7, 8, ... deficiency (−) or duplication (+) chromosomes														
			7 − − − (e)	7 − − (f)	7 − (g)	8 − − (h)	8 + − (i)	8 − (j)	8 + − (k)	8 + (l)	9 − (m)	9 + − (n)	9 + (o)	9 + + (p)	9 + (q)	10 + + (r)	10 + + (s)	10 + + + (t)
15	38	.32								.51								
					.51													
16	26	.44			.12			.12		.12			.12					
		.20		.03					.06						.03			
17	75	.36			.28			.56		.28	.28		.56			.28		
		.30		.06		.06			.12			.12			.06		.06	
		.08	.01				.03							.03				.01
18	8	.44						.04			.04		.04			.04		
		.20				.01						.02					.01	
19	4	.32									.06					.06		
			.01	.09	.91	.07	.03	.72	.18	.91	.38	.14	.72	.03	.09	.38	.07	.01

establishes segregational sterility for the theoretical population at about 40 %.) Application of the Chi square test to Table 10 reveals a very poor fit between the expected and observed numbers ($\chi^2 = ca\ 140$; $N = 6$; $P = \ll .001$). Thus, in so far as the combined four-year total is concerned, the statistical evidence is against the hypothesis of a panmictic breeding system operating in the valley. But this should occasion no great surprise. The evidence must be viewed in the light of the many sources of gross sampling error that doubtless distort the result, for it is abundantly clear, especially from Table 7, that a high degree of introgression is the rule.

Table 9. *Viable Chromosome Recombinants ex Aneuploid Gametes Produced by Crowsnest River Valley Chilocorus*
The chromosome numbers of recombinants are given in the body of the table

Types of single, double and triple deficiency-duplication gametes

	(e) 7 − − −	(f) 7 − −	(g) 7 −	(h) 8 − −	(i) 8 + −	(j) 8 −	(k) 8 − −	(l) 8 +	(m) 9 −	(n) 9 + −	(o) 9 +	(p) 9 + + −	(q) 9 + +	(r) 10 +	(s) 10 + +	(t) 10 + + +
(e) 7 − − −																17
(f) 7 − −													16		17	
(g) 7 −								15			16			17		
(h) 8 − −													17		18	
(i) 8 + − −												17				
(j) 8 −								16			17			18		
(k) 8 + −							16			17						
(l) 8 +			15			16			17							
(m) 9 −								17					18		19	
(n) 9 + −							17					18				
(o) 9 +			16			17			18							
(p) 9 + + −					17											
(q) 9 + +		16		17												
(r) 10 +			17			18			19							
(s) 10 + +		17		18												
(t) 10 + + +	17															

□ $^1/_{12}$ of the recombinant euploid heterozygotes are singles in the 15- and 19-, douples in the 16- and 18-, and triples in the 17-chromosome category.

◇ $^1/_{12}$ of the recombinants are euploid and either single (50%) or triple (50%) heterozygotes.

∩ $^1/_{16}$ of the recombinants are euploid triple heterozygotes.

○ $^1/_{24}$ of the recombinant euploid heterozygotes are doubles in the 16- and 18- and triples in the 17-chromosome category.

Table 10. *Expected and Observed Viable Recombinant Chilocorus by Chromosome Number in the Crowsnest River Valley: 1961—1964*
The a, b, etc. proportions are taken from Tables 8A and B

2n=	14	15	16	17	18	19	20	Total
$(a+b+c+d)^2$	59,390	12,492	6,725	8,690	1,002	411	273	88,983
$(e+f+g\ldots t)^2$		14	22	20	9	2		67
Viable zygotes	59,390	12,506	6,747	8,710	1,011	413	273	89,050*
Number exp.	259	55	30	38	4	2	1	389
Number obs.	226	38	26	75	8	4	12	389

* out of a total of 151,321 zygotes, i.e., *ca* 41% chromosomal sterility.

It is commonly held that interspecific hybridization is an evolutionary dead-end and this is demonstrably so with certain such hybrids in Russia. ZASLAVSKI (1963) has described a case of natural hybridization between two Palaearctic species of *Chilocorus* with results diametrically opposed to those reported here. His two species, *C. bipustulatus* (L.) and *C. geminus* ZASLAVSKI, are narrowly sympatric, overlapping and hybridizing in a zone some 20–30 kilometers wide. In tests, they showed no sexual isolation; reciprocal hybrids developed normally but proved completely sterile both on inbreeding and backcrossing. The hybrids were nevertheless fully viable, showing normal sexual activity and laying eggs. ZASLAVSKI concludes as follows: The sterility of interspecific hybrids, without any other isolation mechanisms, we believe, explains why the margin of the range of the other species, both for *Ch. geminus* and for *Ch. bipustulatus*, is an impassable barrier. (Translation by courtesy of the Foreign Languages Division, Department Secretary of State, Canada.) He visualizes that, with increasing depth-penetrance into the overlap zone, each species from opposite directions is rendered increasingly liable to sterilization by interspecific mating, and at the far margin of the zone has an obligatory zero chance of encountering conspecific individuals and thus of begetting other than sterile progeny.

In the Crowsnest River valley, as we have seen, a similar but one-way infiltration occurs, the *tricyclus* invaders likewise, we must assume, running a decreasing chance of meeting individuals of their own kind with increasing distance from Crowsnest Pass (Figs. 1 and 2). But the Canadian F_1 hybrids, being in some degree fertile and clearly continuously produced, must sooner or later become absorbed into the dominant resident population. The former species, *tricyclus*, must early lose its chromosomal individuality, but the continuity of its gene pool is nevertheless revealed by the persistence in hybrid-derivatives of the diphasics among the autosomes and particularly by the diuturnity of the acrocentric Y chromosome, common even among $2n = 14$ males.

Although mostly inviable recombinants are generated by heterozygotes and despite their resulting population-depressing effect, the act of interbreeding must in the long-run increase the evolutionary plasticity of the population enormously. First, and ignoring the role of crossing-over, simple random recombination of chromosomes from the two species at germ-cell formation assures a potential of 5,103 different chromosomal segregants ($= 3^7 ♀♀ + 3^6 \times 4 ♂♂$). Second, and because of crossing-over (which invariably accompanies meiotic pairing in this genus and even if the single exchanges between chromosome arm-pairs were *strictly* localized), diversity is further enhanced theoretically to more than 148×10^9 ($= 36^7 ♀♀ + 36^7 \times 4 ♂♂$) chromosomally distinct introgressants. Those that thereby gain in adaptive utility, we must assume, will surely

be selected and confer increased genetic versatility upon the eastern community.

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