INTERGENERIC HYBRIDISATION BETWEEN *EREMOPYRUM ORIENTALE* AND *HENRARDIA PERSICA*, AN EXAMPLE OF POLYPLOID SPECIES FORMATION*

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1. Introduction

The tribe Triticeae, Gramineae, includes about 14 genera. Among them *Henrardia* C. E. Hubbard represents a very small genus which contains two annual species, *Hn. persica* (Boiss.) C. E. Hubbard and *Hn. pubescens* (Bertol.) C. E. Hubbard (Hubbard, 1946). The chromosome numbers of both have been reported as $2n = 14$, for the former by Sakamoto and Muramatsu (1965) and for the latter by Bowden (1966). The two representatives have quite a characteristic morphology, different, according to Bor (1960), from other genera of the tribe by the following characters: spikelets 1-2-flowered, awnless, sunk into a jointed fragile spike-axis and closely appressed to it; florets enclosed in collateral glumes; lemmas thin, membranous, 3-5-nerved. Both species are distributed in Turkey (Anatolia) and from there have dispersed eastwards through Armenia and Transcaucasia to Russian Central Asia and southwards to Iraq, Iran, Afghanistan and Baluchistan (Hubbard, *loc. cit.*).

In an attempt to clarify the genetic relationships between this odd looking genus and other genera of the tribe, *Hn. persica* was crossed as either female or male parent with *Aegilops bicornis* Jaub. et Spach, *Ae. caudata* L., *Ae. cylindrica* Host, *Ae. ovata* L., *Ae. sharonensis* Eig, *Ae. squarrosa* L., *Ae umbellulata* Zhuk., *Agropyron tsukushiense* (Honda) Ohwi, *Eremopyrum bonaepartis* (Spreng.) Nevski, *Er. orientale* (L.) Jaub. et Spach, *Heteranthelium piliferum* (Banks et Sol.) Hochst, *Taeniatherum asperum* (Simonkai) Nevski and *Triticum boeoticum* Boiss. From these crosses intergeneric hybrids were obtained so far only in crosses of *Henrardia persica* with *Eremopyrum orientale*. The present report comprises cytogenetic studies of the hybridisation procedure which led to the formation of a new allohexaploid species arising from this combination.

2. Materials and methods

In the present successful intergeneric crosses, a strain of *Eremopyrum orientale* ($2n = 28$; strain no. 7037) was used as the female parent and a strain of *Henrardia persica* var. *glaberrima* (Hausskn.) C. E. Hubbard ($2n = 14$; strain no. 7334) contributed the pollen. These two strains were collected at Ardabil-Tabriz, Iran, by the members of Kyoto University Scientific Expedition to the Karakoram and Hindukush in 1955 (Sakamoto and Muramatsu, 1965).

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The crossing method was as follows. Two lowest florets of each spikelet of *Er. orientale* which served as the female parent were used in 1965 in a greenhouse. Hand-emasculated spikes were enclosed in paraffin-paper bags and were pollinated 2 days later by brushing the stigmas with newly broken anthers of *Hn. persica* serving as the male parent. For the cytological observations the anthers were fixed in Farmer's solution (3 ethanol : 1 acetic acid) and stored in a refrigerator. Chromosome pairing was observed at MI of PMCs using the aceto-carmine squash technique. Microphotographs were taken from temporary preparations. In order to obtain amphiploids, 0·5 per cent. colchicine solution was applied to the basal parts of tillering clones of the hybrids using Sears' method (Sears, 1941).

Voucher specimens of the parental strains, the F_1 hybrid and the amphiploid were placed on file with the Royal Botanic Gardens, Kew, and the National Science Museum, Tokyo.

3. Results

From the pollination of 219 florets of *Eremopyrum orientale* with pollen of *Henrardia persica* 47 seeds were obtained. All were sown and gave seedlings, among them 25 true hybrids and 10 non-hybrids due to incomplete hand-emasculation.

(a) *Morphology of the parents and F_1 hybrids*

Several quantitative characters of the parents and the F_1 plants are listed in table 1. The average dates of first flowering of *Er. orientale*, *Hn. persica* and F_1 plants in the greenhouse were respectively 1st May, 11th May and 30th April. Thus the F_1 plants started to bloom as early as the *Eremopyrum* parent.

Table 1

Quantitative characters

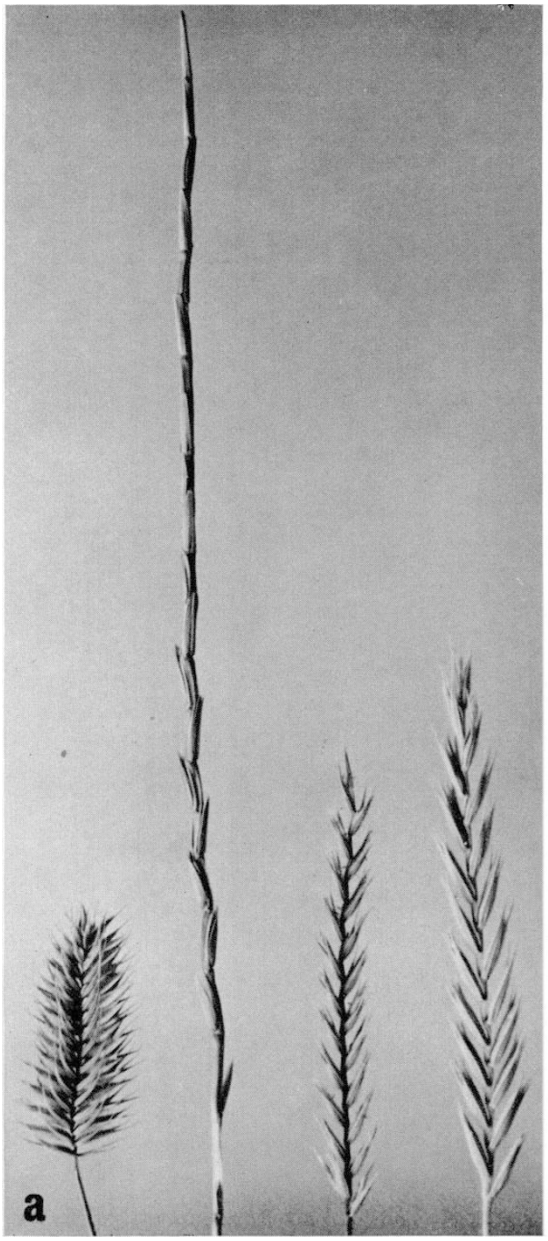
Characters	*Eremopyrum orientale*	*Henrardia persica*	F_1
Average date of first flowering	1st May	11th May	30th April
No. of tillers	56·7	57·7	78·0
Culm length (cm.)	41·3	49·7	44·7
Length of top internode (cm.)	16·0	7·9	16·6
Length of flag leaf (cm.)	5·3	9·8	5·6
Length of spike (cm.)	3·6	17·5	7·4
No. of spikelets per spike	22·8	18·9	21·3

A marked heterotic increase in the number of tillers of the F_1 plants was observed, while the length of culms and spikes was intermediate between the parents. On the contrary, the length of the top internode and flag leaf and the number of spikelets per spike were similar to those of the *Eremopyrum* parent. The highest internode below the spike of the F_1 plants was puberulent like in *Er. orientale*.

As plate I, fig. *a* clearly shows, the spike shape of the F_1 was intermediate though clearly nearer to *Eremopyrum*, while the spikelets were distinctly of *Eremopyrum* type (plate I, fig. *b*). The glumes of *Er. orientale* are narrowly linear-acuminate, hispid, keeled and awned. Those of *Hn. persica* are broad, glabrous, keelless and awnless. The F_1 glumes were of *Eremopyrum* type but the hairs were very short. The lemma of *Er. orientale* is narrowly elliptic-acuminate as long as the glumes, indurated, hispidulous and shortly awned.

Chromosome pairing of *Eremopyrum orientale*, *Henrardia persica*, their F_1 hybrid and amphiploid and abnormal pollen formation of the F_1 ($\times 750$).

Fig. *a*.—Diakinesis of *Er. orientale* with 14 bivalents.
Fig. *b*.—Metaphase I of *Hn. persica* with 7 bivalents.
Fig. *c*.—Metaphase I of the F_1 with 21 univalents. (Chromosome derived from *Hn. persica* are indicated by arrows.)
Fig. *d*.—Metaphase I of the F_1 with one bivalent and 19 univalents.
Fig. *e*.—Metaphase I of the F_1 with two bivalents and 17 univalents.
Fig. *f*.—Abnormal pollen formation of the F_1.
Fig. *g*.—Metaphase I of the amphiploid with 21 bivalents.
Fig. *h*.—Metaphase I of the amphiploid with 20 bivalents and two univalents.
Fig. *i*.—Metaphase I of the amphiploid with 19 bivalents and four univalents.



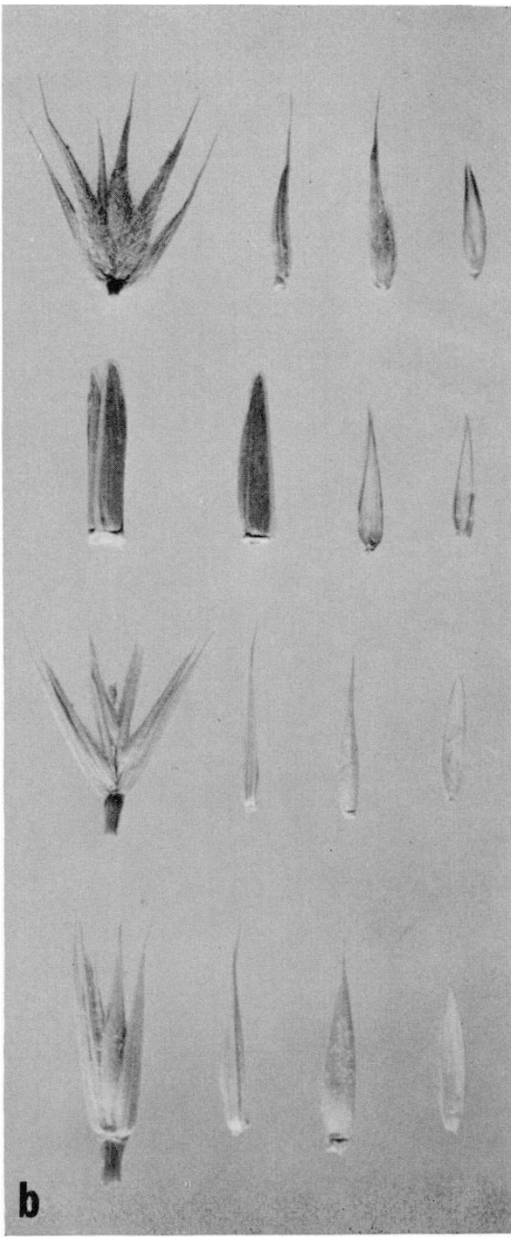
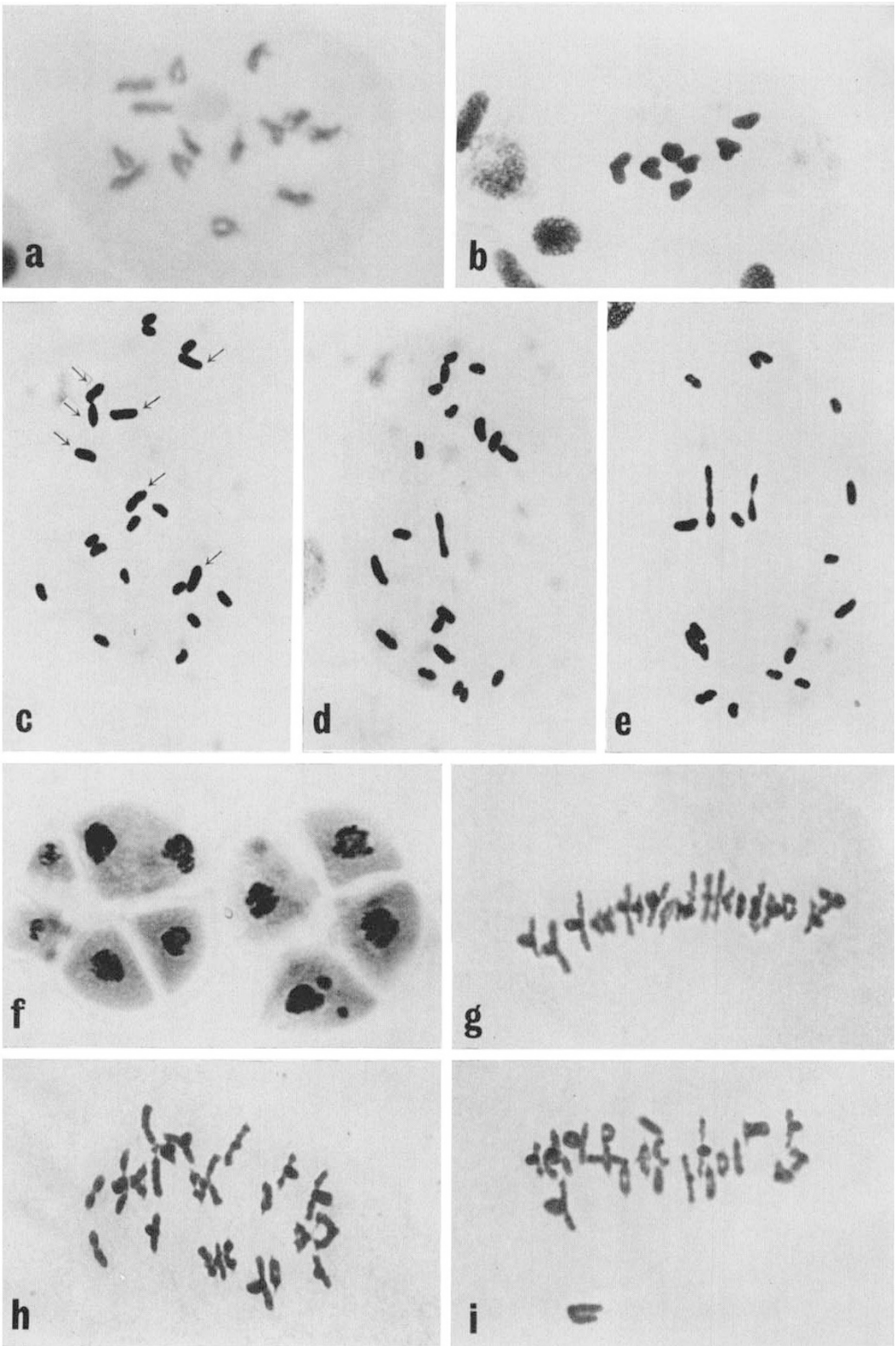


Plate I

FIG. *a*.—Spikes. From left to right: *Eremopyrum orientale*, *Henrardia persica*, F_1 hybrid, the amphiploid (×0·85).

FIG. *b*.—Spikelet, glume, lemma and palea. From top downward: *Eremopyrum orientale*, *Henrardia persica*, F_1 hybrid, the amphiploid (×2·5).


a
b
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On the contrary, that of *Hn. persica* is broad, membranous, 3-5-nerved, glabrous and awnless. In the F_1 the *Eremopyrum* type was predominant. The palea of *Er. orientale* is hard, 2-keeled and hairy between the keels while that of *Hn. persica* is membranous, but also 2-keeled and hairy. The F_1 plants were intermediate between the parents (plate I, fig. *b*). Disarticulation of ripe spikelets of *Er. orientale* is of wedge-type, *i.e.* at every lower joint of the rachis. However, that of *Hn. persica* is of barrel-type (*i.e.* brittle at every upper joint). The F_1 showed the wedge-type disarticulation of the *Eremopyrum* parent.

(b) *Cytology and fertility of F_1 hybrids*

Chromosome pairing at MI of the parents, F_1 and amphiploids is listed in table 2 and plate II, figs. *a-i*. Of 2888 PMCs examined, 1894 (65·6 per

TABLE 2

Chromosome pairing at MI of PMCs

Strains	Chromosome pairing III	II	I	No. of cells observed
Parents				
Eremopyrum orientale	—	14	—	42 (100·0%)
Henrardia persica	—	7	—	485 (94·8)
	—	6	2	26 (5·1)
	—	5	4	1 (0·1)
Total				512
F_1 hybrid	—	4	13	3 (0·001)
	—	3	15	21 (0·007)
	—	2	17	169 (5·9)
	1	—	18	2 (0·007)
	—	1	19	799 (27·7)
	—	—	21	1894 (65·6)
Total				2888
Amphiploid	—	21	—	86 (32·8)
	—	20	2	99 (37·8)
	—	19	4	48 (18·3)
	—	18	6	20 (7·6)
	—	17	8	6 (2·3)
	—	16	10	3 (1·1)
Total				262

cent.) showed 21 univalents (plate II, fig. *c*). One bivalent + 19 univalents and two bivalents + 17 univalents were found, respectively, in 799 (27·7 per cent.) and 169 (5·9 per cent.) cells. Cells with more than three bivalents were seldom observed. One trivalent was found in two cells only. Average chromosome pairing per PMC of the F_1 showed 0·01 trivalent, 0·42 bivalent and 20·20 univalents. Bivalents ranging from one to four per cell were all terminally associated (plate II, figs. *d* and *e*). Somatic chromosomes of *Henrardia persica* are larger than *Eremopyrum*'s (Sakamoto and Muramatsu, 1965). Plate II, fig. *c* clearly shows at MI of the F_1 seven larger univalents of *Hn. persica* (indicated by arrows) and 14 smaller ones of *Eremopyrum orientale*. It is interesting that the seven larger chromosomes of *Henrardia* retain the same larger dimensions in the *Eremopyrum* cytoplasm, alien to them.

Abnormal pollen formation in the F_1 was often observed as shown in plate II, fig. *f*. 6030 pollen grains of F_1 plants were examined. The majority were abortive and only 26 (0·44 per cent.) stained by diluted aceto-carmine. The anthers were non-dehiscent. Complete seed sterility was observed in self- and open-pollinated spikes.

(c) *The amphiploids*

Applying 0·5 per cent. colchicine solution to the basal parts of tillering clones of the F_1, 150 well-developed seeds were obtained from 67 spikes of four treated plants. Out of 62 seeds sown in 1966, 58 germinated normally and gave 52 vigorous amphiploid plants; six died before heading.

The morphological features of the colchicine-induced amphiploids were similar to those of the F_1 plants but most of the plant parts showed larger dimensions than the F_1 (plate I, figs. *a* and *b*). The average date of the first flowering in the greenhouse was 14th May. Disarticulation of the ripe spikelets was of wedge-type like in *Er. orientale* and in F_1.

Chromosome pairing at MI of the PMCs of the amphiploids is shown in plate II, figs. *g-i* and table 2. Out of 262 cells, about one-third (32·8 per cent.) had 21 bivalents (plate II, fig. *g*), one-third (37·8 per cent.) showed 20 bivalents and two univalents and the remaining cells contained 16-19 bivalents per cell. No trivalent or higher association was found. Average chromosome pairing was 20·04 bivalents and 2·24 univalents per PMC. Of 2430 pollen grains examined 1839 were stainable. Therefore, pollen fertility was assessed at 75·7 per cent. Self- and open-pollinated seed fertilities were 48·3 per cent. and 81·4 per cent., respectively.

4. Discussion

In his taxonomical revision of taxa belonging to two very similar genera *Lepturus* and *Pholiurus* sensu Camus (1922), Hubbard (1946) recognised that two species of *Pholiurus*, *Ph. persicus* (Boiss.) A. Camus (= *Lepturus persicus* Boiss.) and *Ph. pubescens* (Bertol.) A. Camus (= *Rottboellia pubescens* Bertol.), had the following characters: ovary hairy at the apex, lodicule hairy, lemma three or more nerved and seed longitudinally grooved with simple starch grains in the endosperm. These characters have been regarded to be of diagnostic value in distinguishing the tribe Triticeae from other Gramineae tribes. On this basis Hubbard separated the two taxa from other *Pholiurus* taxa and established a new genus *Henrardia* C. E. Hubbard. The nomenclatural transfer was as follows: *Henrardia persica* (Boiss.) C. E. Hubbard and *Hn. pubescens* (Bertol.) C. E. Hubbard.

To elucidate the genetic relationships between the new genus *Henrardia* and other genera of the Triticeae tribe, the present author carried out extensive intergeneric crosses of *Henrardia persica* with *Aegilops*, *Agropyron*, *Eremopyrum*, *Heteranthelium*, *Taeniatherum* and *Triticum*. Among them only the hybrid between *Eremopyrum orientale* and *Hn. persica* succeeded. This result provided an important cytogenetic evidence for the correctness of Hubbard's taxonomical revision of the genus *Henrardia* and at the same time further strengthened the importance of intergeneric hybridisation as a reliable means for distinguishing the differences between morphological parallelism among unrelated taxa and morphological diversity among related ones.

Crossability between *Er. orientale* and *Hn. persica* was 11·4 per cent. calculating from the number of hybrid plants produced and number of florets pollinated. Although, as mentioned previously, the genus *Henrardia* due to its odd phenotypic traits is taxonomically quite distinct from other genera of the tribe Triticeae, the percentage of crossability shows indisputably a high genetic or may be mainly cytoplasmic compatibility between *Eremopyrum* and *Henrardia.*

As mentioned above, spike length of the F_1 plants was intermediate between the parents but clearly nearer to *Eremopyrum* and various parts of F_1 spikelets were of *Eremopyrum* type as was observed also in *Er. orientale* × *Aegilops squarrosa* (Sakamoto, 1968). This may partly be the effect of the dosage, *i.e.* two genomes of *Er. orientale vs.* one genome of *Hn. persica* or *Ae. squarrosa* in the F_1 hybrids.

Lack of bivalent pairing in 66 per cent. of the F_1 PMCs, 0·42 bivalent per cell as the average in the F_1 and high bivalent pairing, 20·04 per cell, of

TABLE 3

Average chromosome pairing and pollen- and seed-fertilities in three intergeneric F_1 hybrids and their amphiploids

Cross combinations (♀×♂)	Average chromosome pairing IV	III	II	I	Pollen fertility (%)	Seed fertility (%)*
Eremopyrum orientale (4×) × *Agropyron tsukushiense* (6×):						
F_1 (5×)	—	—	0·5	34·0	0·003	0
Amphiploid (10×)	—	—	29·4	11·2	40·2	5·5
Eremopyrum orientale (4×) × *Henrardia persica* (2×):						
F_1 (3×)	—	0·0	0·4	20·2	1·0	0
Amphiploid (6×)	—	—	20·0	2·2	75·7	48·3
Eremopyrum bonaepartis (2×) × *Hordeum* sp. (4×):						
F_1 (3×)	0·0	0·0	5·5	10·0	1·0	0
Amphiploid (6×)	0·2	0·3	17·8	4·9	56·3	34·1

* Self-pollinated seed fertility.

the amphiploids indicate the two genomes of *Er. orientale* and that of *Hn. persica* are not homologous. Rare occurrence of trivalents in F_1, absence of multivalent association in F_1 as well as in the amphiploid, and formation of loosely connected terminally associated bivalents in F_1 also suggest lack of segmental homology among the chromosome complements of the three genomes of the two distantly related genera, two genomes of *Eremopyrum* and one of *Henrardia.*

From my cytogenetic studies of the genus *Eremopyrum* it is known that *Er. orientale* is an allotetraploid derived from amphidiploidisation between diploid *Er. distans* and diploid *Er. triticeum* (Sakamoto, 1967). Thus, the colchicine-induced hexaploid amphiploid plants have three different genomes *Er. distans*, *Er. triticeum* and *Hn. persica* respectively. The genera *Eremopyrum* and *Henrardia* are quite different from each other. The hybrid could be established only as an amphiploid. Similar amphiploids were recently produced in course of my study in two combinations of intergeneric hybridisation, *i.e. Eremopyrum orientale* ($2n = 28$) × *Agropyron tsukushiense* ($2n = 42$) and *Er. bonaepartis* ($2n = 14$) × *Hordeum* sp. ($2n = 28$) as shown

in table 3 (Sakamoto, unpublished). In this table the average chromosome pairing at MI of PMCs and pollen- and seed-fertilities of the F_1 hybrids and their amphiploids are summarised. Low pollen- and seed-fertilities of the amphiploid between *Er. orientale* and *Ag. tsukushiense* could be interpreted as the result of unexpected high frequency of univalent formation at MI. The average bivalent pairing, 5·5, observed in F_1 hybrid between diploid *Er. bonaepartis* and tetraploid *Hordeum* sp. indicate autosyndesis between chromosomes derived from the *Hordeum* parent. In these three amphiploids represented in table 3 quite different genomes were involved. In the first combination two genomes of *Eremopyrum* and three of *Agropyron* were involved. In the second two genomes of *Eremopyrum* and one of *Henrardia* and in the third case one genome of *Eremopyrum* and two of *Hordeum* were combined. However, all genomes could act as they do in *Eremopyrum* cytoplasm. Many intergeneric amphiploids in the tribe Triticeae were reported, for instance, such combinations as *Agropyron* × *Hordeum*, *Elymus* × *Sitanion*, *Secale* × *Triticum*, *Triticum* × *Aegilops*. In the cases described above, no disharmony could be observed between cytoplasm and introduced alien genomes. Even the larger size of *Henrardia* chromosomes was realised in the cytoplasm of *Eremopyrum* which has small chromosomes.

5. Summary

1. *Henrardia* is one of the very small genera in the tribe Triticeae represented by two annual species, *Hn. persica* and *Hn. pubescens*. Morphologically this genus is different from the other members of the tribe. In an attempt to clarify genetic relationships of this genus with other genera of the tribe, a strain of *Hn. persica* var. *glaberrima* was crossed with various species of *Aegilops*, *Agropyron*, *Eremopyrum*, *Heteranthelium*, *Taeniatherum* and *Triticum*.

2. From these crosses F_1 hybrids were produced only when *Er. orientale* was used as the female parent. Morphological characters of the F_1 hybrids were of *Eremopyrum* type. Average chromosome sairing per PMC of the F_1 was 0·01 trivalent, 0·42 bivalent and 20·20 univalents. Bivalents ranging from one to four were all terminally associated. No genomic homology was found between *Er. orientale* and *Hn. persica*. Complete sterility was observed.

3. Applying 0·5 per cent. colchicine solution to the basal parts of tillering clones of the F_1, many well-developed seeds were obtained. From them vigorous amphiploids were grown. Average chromosome pairing per PMC of the amphiploids was 20·04 bivalents and 2·24 univalents. Pollen and self-pollinated seed fertilities were 75·5 per cent. and 48·3 per cent., respectively.

4. The successful production of those intergeneric hybrids provides important evidence for the correctness of Hubbard's (1946) taxonomical treatment of the genus *Henrardia* and at the same time further strengthens the importance of intergeneric hybridisation as a reliable means for distinguishing the differences between morphological parallelism among unrelated taxa and morphological diversity among related ones.

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