Natural Hybridization between *Aegilops sharonensis* and *Ae. longissima*: A Morphological and Cytological Study¹

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Introduction

Evolutionists have come to appreciate the significance of interspecific hybridization in consideration of problems of variation and evolution in plants (for reviews see Anderson 1953, Anderson and Stebbins 1954, Stebbins 1959). But most of the evidence concerning natural hybridization in plants consists of morphological comparisons of variation patterns in populations, now mainly carried out by Anderson's (1949) technique of pictorialized scatter diagrams.

In the present work an attempt was made to supplement such a morphological analysis with cytological evidence. This report deals with natural hybridization between two diploid ($2n=14$) *Aegilops* species native to Israel, namely *Ae. sharonensis* Eig and *Ae. longissima* Schweinf. et Muschl. When hybrid swarms between these two species were encountered, they were of attraction to us since these grasses were known to differ from one another in a reciprocal translocation (Tanaka 1955). They therefore afforded an opportunity to verify conclusions based on morphological analysis by use of a chromosomal marker.

Examinations in natural populations

1. Material examined

Both *Ae. sharonensis* and *Ae. longissima* are widely spread in Israel over the sandy soil belt of the mediterranean coastal plain. In the Sharon Plain, i. e. between Tel-Aviv and Haifa, they are many times found growing side by side. As already reported by Eig (1929) these two species are, as a rule, easily distinguishable from one another, both morphologically and ecologically. Stand of *Ae. sharonensis* usually occupy consolidated sand dunes and well-drained, somewhat loose sandy loams. *Ae. longissima* shows relatively wider ecological preferences, as well as a wider geographical distribution. In the Sharon Plain it occupies mainly calcareous sandstone soils, and somewhat siltier or ill-drained sandy loams. The two species are also seasonally separated: *Ae. longissima* flowers 2-3 weeks later than *Ae. sharonensis*.

¹ The data presented are taken from an M. Sc. thesis submitted by the first author to the Department of Botany, The Hebrew University, Jerusalem.

Repeated observations in the Sharon Plain showed us that, while this description holds good for areas which remained ecologically rather stable, this is not always the case in sites which have been recently drastically disturbed by man, i. e. edges of cultivation, roadsides etc. In such places

Fig. 1. Representative sample of spikes from plants encountered in the Ra'anana hybrid swarm. 1, typical *Ae. longissima* spike. 8, to 9, typical *Ae. sharonensis* spike. 2 to 7, intermediate forms.

—and when the two species grow side by side—blurring of specific boundaries is common. Series of intermediates and recombinants are frequently met with here, and populations of both species exhibit characteristic variation

patterns of introgressive hybridization. In several such drastically disturbed places we found fully developed hybrid swarms. The following two sites were chosen for examination in spring 1957.

Ra'anana hybrid swarm: This hybrid population was found on the Petah-Tiqva-Haifa road, approximately 5 km north of Ra'anana. The station is located in the middle of the red sandy loam belt of the Sharon Plain. It consisted of a mixed stand of *Ae. sharonensis*, *Ae. longissima* and appreciable numbers (up to 20%) of intermediates and recombinants (a representative sample is shown in figure 1). The hybrid population was concentrated mainly on a newly dug roadside ditch and spread also to the edge of an adjacent cultivated field. In contrast, relatively pure populations of *Ae. sharonensis* and *Ae. longissima* colonized the neighbouring undisturbed sandy loam hills. The ecological difference was indeed sharp; only a few meters away from the road-ditch not even a single clearly intermediate plant could be detected. The roadside population was examined.

Tsahala hybrid swarm: A hybrid population similar to the Ra'anana swarm was found growing at the edge of Tel-Aviv-Hertzeliya road, approximately 2 km north of the Yarkon river. It consisted again of a mixed population of *Ae. sharonensis* and *Ae. longissima* accompanied by a whole array of intermediates and recombinants. Here, too, the hybrid swarm was confined mainly to a newly dug strip of an underground telephone cable. The undulating red sandy loam areas in the vicinity were occupied by dense stands of both species. But in several places, particularly in previously ploughed patches, highly introgressed populations of both species were noted. In the Tsahala station a random sample of plants was collected from the swarm at the roadside.

Control populations: For comparison with the hybrid swarms, pure populations of *Ae. sharonensis* and *Ae. longissima* were also examined. For this comparison we selected pure stands, i. e. populations which were not growing in close proximity to the second *Aegilops* species. A random sample of pure *Ae. sharonensis* was taken 1 km east of Caesarea, Sharon Plain, where it was separated by a distance of at least 1 km from the nearest *Ae. longissima* plants. One population of pure *Ae. longissima* was sampled at Rehovot, again several kilometers away from the nearest *Ae. sharonensis* stand. A second *Ae. longissima* population was sampled further south, at Gilat, approximately 17 km northwest of Beersheba.

2. Morphological analysis

The Anderson technique of pictorialized scatter diagrams was employed to obtain a clear picture of the patterns of variation found in the various populations investigated. Fig. 2 represents the results obtained from an examination of the control populations at Rehovot and Caesarea as well as from that of artificial F_1 and F_2 generations. Only one population of pure *Ae. longissima* is represented in the scatter diagram, since the variation

Fig. 2. Pictorialized scatter diagrams comparing samples taken from pure populations of *Ae. sharonensis* (Caesarea) and *Ae. longissima* (Rehovot) with artificially produced F₁ and F₂ plants.

Fig. 3. Pictorialized scatter diagrams of samples taken from the two hybrid swarms.

picture obtained in the second control population at Gilat resembled that of Rehovot. Fig. 3 represents the hybrid populations of Ra'anana and Tsahala. Data from the various populations examined are also represented in a condensed form, in hybrid index histograms, in Fig. 4.

The following traits were employed for the morphological examination of the various populations sampled. For further details on morphological differences between the two species consult Eig (1929) pp. 77-78.

Length of spike: While rachis internodes in *Ae. longissima* are relatively long, causing an appearance of a cylindrical or single-rowed ear, the internodes in *Ae. sharonensis* are shorter, particularly in the central part of the spike. The ear appears therefore imbricated or 2-rowed. The ratio: number of spikelets in ear over length of ear (exclusive of awns) was employed to express this difference (abscissae in Figs. 2 and 3).

Glume-rachis internode relationship: *Ae. longissima* and *Ae. sharonensis* differ from one another in the relative length of the spikelet glumes, as compared with the length of their respective rachis internodes. The spikelet situated at $\frac{2}{3}$ the height of the ear was employed for these measurements. The ratio: length of the rachis internode over length of the lower glume in the corresponding spikelet was employed (ordinates in Figs. 2 and 3).

Awns on lateral spikelets: The lateral spikelets of the cylindrical ear of *Ae. longissima* are devoid of awns. In contrast, *Ae. sharonensis* (excepting its rare var. *mutica*) is characterized by development of awns on most of the lateral spikelets. A scale of three grades was employed for expression of this trait.

Structure of lemma tip (in the lemma-awn transition area): In *Ae. longissima*'s terminal spikelet, the tip of the lemma passes gradually into the prominent awn. The awn is accompanied at its base by two narrow, long teeth. In *Ae. sharonensis* the lemma passes abruptly into a relatively

Explanation of symbols used in scatter diagrams for Figs. 2-3.

1. <i>Awns on lateral spikelets</i> :	4. <i>Cross section through awn</i>
○ lacking	○ flat triangle
◊ present in upper half of spike	◊ intermediate
◌ spikes fully awned	◌ equilateral triangle
2. <i>Awn base</i>	5. <i>Disarticulation of rachis</i>
○ <i>longissima</i> -like	○ in 1-2 points
◊ intermediate	◊ in 3-6 points
◌ <i>sharonensis</i> -like	◌ at every node
3. <i>Shape of glume apex</i>	6. <i>Chromosomal constitution</i>
○ prominent teeth	○ plants homozygous (7 II)
◊ intermediate	◊ plants heterozygous,
◌ blunt teeth	(5 II+1 IV)

thin awn, accompanied by two broad, short teeth (Fig. 5, bottom). Plants were scored as to awn structure in their terminal spikelet. Three grades were adopted.

Fig. 4. Hybrid index histograms of the various populations.

shape of such a section is an equilateral triangle (Fig. 5, top). Three grades were recognized.

Disarticulation of the rachis: In *Ae. longissima* the cylindrical ear disarticulates only at 1 or 2 points at the lower part of the spike; thus most of the spikelets form a single, long dispersal unit. In *Ae. sharonensis*, the rachis disarticulates practically at every spikelet base and the fruit dispersal unit consists of a single spikelet. A scale of three grades, representing the degree of brittleness of the ear rachis, was employed.

Shape of glumes: In *Ae. longissima* the glume apex in the lateral spikelets is furnished with two teeth with a deep sinus between them. In *Ae. sharonensis* it is characterized by two short, broad teeth separated by a shallow sinus, or it is merely emarginate (Fig. 5, center).

Cross section through the base of the awn: In *Ae. longissima* a cross section through the base of the awn of the terminal spikelet reveals a flat isosceles triangle. In *Ae. sharonensis* the

3. Cytological observations

The acetocarmine smear technique was employed for the examination of microsporocytes in meiosis. Anthers were fixed in the field in a 1:3 aceto-alcohol solution.

The difference in the reciprocal translocation between *Ae. sharonensis* and *Ae. longissima* (Tanaka 1955) was made use of in the cytological detection of hybridization in natural populations. Plants were checked during diakinesis or metaphase I as to whether they were chromosomically homozygous or structurally heterozygous. The results of the cytological observations in samples from the various populations are presented in Table 1.

In the Ra'anana hybrid swarm each plant sampled was examined both morphologically and cytologically. The results of this combined examination are also

Ae. longissima intermediate Ae. sharonensis
condition

Fig. 5. Morphological characters used for scoring *Ae. longissima* vs *Ae. sharonensis*. Top row: cross section through the base of the awn. Central row: shape of glume's apex. Bottom row: structure of lemma's tip.

Table 1. Results of cytological examinations of plants from various natural populations, as well as of artificial F_1 and F_2 generations

Population	Total number of plants examined	Homozygous plants (7 II)	Heterozygous plants (5 II+1 IV)
Hybrid swarm Ra'anana	64	47	17
Hybrid swarm Tsahala	17	15	2
<i>Ae. sharonensis</i> Caesarea	18	18	—
<i>Ae. longissima</i> Rehovot	10	10	—
<i>Ae. longissima</i> Gilat	10	10	—
F_1 generation	20	—	20
F_2 generation	42	18	24

represented in the scatter diagram in Fig. 3 (downward pointing bar indicates chromosomal constitution). In the Tsahala hybrid swarm the cytological examination was not carried out in the same plants which served for morphological analysis. Here, 17 intermediate-looking plants were selected for cytological observations. In each of the control populations, two random samples were taken, one for cytological and one for morphological examination.

Fig. 6. Microsporocyte in metaphase I, showing 5 bivalents and a translocation chain of 4 chromosomes.

In both hybrid swarms, as can be seen from Table 1, a considerable number of plants showed five bivalents and a translocation quadrivalent (see also Fig. 6). In contrast, no translocation

configurations were found in the control pure populations examined.

Artificial hybrids

Artificial crosses between *Ae. sharonensis* and *Ae. longissima* were made in 1957, and F_1 and F_2 hybrids were raised in Jerusalem in consequent years for comparison with the natural hybrid swarms. The appearance of F_1 plants was intermediate between that of the two parental species and resembled plant No. 4 in Fig. 1. Wide segregation was already apparent in F_2 plants. Results of the morphological examination of artificial hybrids are presented for comparison in Fig. 2.

A number of F_1 plants were examined cytologically (see Table 1), and it was confirmed that all were heterozygous for the translocation. It is also noteworthy that the set in the F_1 plants was normal, and that pollen abortion was only about 15% in spite of the translocation ring.

In the F_2 generation 42 plants were analyzed. 24 were found to be translocation heterozygotes, while 18 others showed normal meiosis, i. e. seven bivalents.

Discussion

As already stated in the introduction, the translocation distinguishing *Ae. sharonensis* from *Ae. longissima* rendered an opportunity for a cytological verification of the process of hybridization. Our cytological finds are in full accord with the morphological examination. The occurrence of an appreciable number of translocation heterozygotes in populations recognized morphological-

ly as hybrid swarms, and their absence in pure populations, verify the hybridization picture. Thus, this case demonstrates the soundness of conclusions reached on the basis of a morphological analysis (by Anderson's method).

One should also stress the fact that *Ae. sharonensis* and *Ae. longissima* are usually well differentiated from one another in nature, in spite of their overlapping distribution. Contrary to doubts expressed by several workers (e. g. Kihara 1954, Riley, Unrau and Chapman 1958), we are inclined to accept Eig's (1929) recognition of these forms as two separate species. The sporadic occurrence of hybridization and intermediate forms between them, as well as the fertility of F_1 hybrids, does not call for a revision in this concept. Hybrids are encountered almost exclusively in places recently disturbed by man; they therefore actually indicate that these two taxa are apparently effectively separated by ecological isolation. Hybrids and hybrid derivatives, even if occasionally formed, are apparently ill-adapted to survive in stable, natural habitats and have, most likely, only a precarious existence.

Attention should be also drawn to the problem of stocks of seed of *Ae. sharonensis* and *Ae. longissima* used in cytogenetic laboratories. During collection trips, seed is often collected near roadsides where hybrid derivatives are relatively common. From his photographs one might suspect that Roy (1959) started his work on *Ae. longissima* with material already highly introgressed with *Ae. sharonensis*. This is apparently the explanation why Roy did not encounter a translocation configuration in his artificial hybrids between the two species.

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Summary

Natural hybridization between *Ae. sharonensis* and *Ae. longissima* was found to occur in the Sharon Plain of Israel, but to be confined to disturbed habitats. Hybrid swarms were examined morphologically by means of Anderson's technique of population sampling and were found to exhibit characteristic hybrid variation patterns. Since the species concerned differ in a reciprocal translocation, the hybrid nature of the populations investigated could be assessed from the presence of translocation heterozygotes. These observations have also been supplemented by an examination of artificial F_1 and F_2 hybrids.

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