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# Allozyme variation in Transcaucasian populations of *Aegilops squarrosa*

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Genetic variation at 27 enzyme loci was studied in 24 Transcaucasian populations of *Aegilops squarrosa*: 12 ssp. *eusquarrosa* and 12 ssp. *strangulata* populations. Most of the genetic variation was among populations: $G_{ST}$ accounted for 0.67 and 0.64 for ssp. *eusquarrosa* and ssp. *strangulata*, respectively. The *Acph1*, *Est2*, *Est5*, *Got1* and *Got2* loci were found to be involved in the adaptive process of *Ae. squarrosa* subspecies divergence. Allele variation at the *Ep* locus is most likely to be neutral. The remaining 21 enzyme loci are under purifying selection of different intensities.

**Keywords:** *Aegilops squarrosa*, allozymes, genetic structure, natural selection, speciation.

## Introduction

*Aegilops squarrosa* L. is a diploid self-pollinating goatgrass species which has contributed the D genome to common wheat (*Triticum aestivum* L.). Its importance as a potential donor of desirable genes for the improvement of cultivated wheats has aroused interest in investigating genetic variation in this species. Studies of variation among geographically diverse accessions of *Ae. squarrosa* from the world germ-plasm collections have been carried out using molecular markers, such as allozymes (Jaaska, 1980, 1981, 1984; Lagudah & Halloran, 1989; Dudnikov & Goncharov, 1993), storage proteins (Lagudah & Halloran, 1988) and DNA markers (Lubbers *et al*., 1991). Information on the extent of genetic variation in *Ae. squarrosa* and the general geographical patterns of this variation through the species range have been obtained. Two problems have been identified, which are essential for understanding processes of evolution and also for the development of an appropriate and efficient conservation strategy: (i) *Ae. squarrosa* intraspecies divergence; and (ii) the genetic structure of *Ae. squarrosa* populations. Such studies need plant material collected in primary habitats of *Ae. squarrosa* which are representative of gene frequencies in local populations of the species.

Despite a long history of studies on *Aegilops* systematics, the constitution of subspecies in *Ae. squarrosa* is not clear. Eig (1929) identified two subspecies: ssp. *strangulata* Eig with a markedly moniliform appearance of the spike, and ssp. *eusquarrosa* Eig ( = ssp. *tauschii* Hammer) with a cylindrical type of spike. In reality, these two typical forms are connected by a continuous range of intermediate forms. This presents a problem for researchers as a considerable number of plants can not be unambiguously assigned to either of the subspecies (Jaaska, 1981; Lagudah & Halloran, 1988). Therefore, distinguishing between *Ae. squarrosa* subspecies is largely subjective. Tanaka (1983) assigns to ssp. *strangulata* only plants with a sharply defined moniliform spike, which occupy the narrow belt along the south-eastern Caspian shore near Gorgan in Iran. According to Jaaska (1981), ssp. *strangulata* is morphologically more variable and inhabits eastern Armenia, Azerbaijan, Nakhitshevan, Northern Iran and Western Kopet-Dag in Turkmenistan. Zhukovsky (1928) divided *Ae. squarrosa* into three subspecies.

The objectives of this work, besides the study of *Ae. squarrosa* intraspecific differentiation and the genetic structure of *Ae. squarrosa* populations, were to investigate the nature of allozyme variation in *Ae. squarrosa* populations and the role of allozymes in the process of speciation.

## Materials and methods

Plant material was collected in 1989 in Armenia and Azerbaijan, and in 1990 in Dagestan (Table 1, Fig. 1) in *Ae. squarrosa* primary habitats. *Aegilops*

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*squarrosa* is distributed in hilly or mountainous regions where its primary habitats are dwarf-shrub steppe-like formations, usually just below the edge of a forest belt. In such habitats *Ae. squarrosa* makes up about 10 per cent of the herbage. Populations in secondary habitats were not used for seed collection. Seeds of *Ae. squarrosa* were collected randomly as separate spikes from mature plants at each of the 20 locations. One seed from each spike was germinated and analysed electrophoretically.

Leaf tissue of 2–3-week-old green plants was homogenized as described by Hart & Langston (1977). Sixteen enzyme systems were used: aconitate hydratase (ACO, EC 4.2.1.3), acid phosphatase (ACPH, EC 3.1.3.2), aldolase (ALD, EC 4.1.2.13), catalase (CAT, EC 1.11.1.6), endopeptidase (EP, EC 3.4.21–24.-), esterase (EST, EC 3.1.1.2), glyceraldehyde-3-phosphate dehydrogenase (GAPD, EC 1.2.1.12), glutamate dehydrogenase (GDH, EC 1.4.1.2), glutamatic-oxaloacetatic transaminase (GOT, EC 2.6.1.1), general protein (GP, EC 4.1.1.39?), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9), leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), NADH diaphorase (NADHD, EC 1.6.4.3), phosphoglucomutase (PGM, EC 2.7.5.1) and shikimate dehydrogenase (SKDH, EC 1.1.1.25).

A polyacrylamide gel electrophoretic system with 0.25 M Tris-0.1 M HCl gel buffer (Jaaska, 1981) was used for EP and GOT. Starch gel electrophoresis was performed horizontally on 12 per cent starch gels cooled with ice packs. A Tris-EDTA-maleic acid system, pH 7.4 (Brown *et al*., 1978) was used for GP, LAP and NADHD. LiOH-borate, pH 8.3, was used for ACPH, ALD, CAT, EST and GPI; and 0.02 M histidine-citrate, pH 7.0, was used for the other enzymes (Gottlieb, 1981).

ALD, CAT, GDH, GOT, GP, GPI, LAP, MDH, NADHD and PGM were stained as in Brown *et al*. (1978). ACO was stained as in Chenicek & Hart (1987), EP was stained as in Tang & Hart (1975), EST was stained as in Jaaska (1980), GAPD was

**Table 1** Locations where *Aegilops squarrosa* plant material was collected. Ssp. *eusquarrosa* and ssp. *strangulata* populations are designated as 'e' and 's', respectively

| Location number | Population | Locality | Longitude[1] | Latitude[1] | Altitude (m) |
|---|---|---|---|---|---|
| | | **Dagestan** | | | |
| 1 | 1e | Vicinity of Gedzhuh village | 48.07 | 42.09 | 120 |
| 2 | 2s | Vicinity of Ersy village | 48.04 | 42.07 | 200 |
| 3 | 3s | Vicinity of Darvag village | 48.05 | 42.03 | 300 |
| 4 | 4e, 4s | Vicinity of Hily village | 48.07 | 41.95 | 450 |
| 5 | 5e | Vicinity of Maraga village | 48.11 | 41.94 | 420 |
| 6 | 6e, 6s | Vicinity of Rukel village | 48.24 | 41.98 | 380 |
| 7 | 7e | The western foot of hill '336' | 48.26 | 41.98 | 160 |
| 8 | 8s | The top of hill '336' | 48.28 | 42.01 | 350 |
| 9 | 9e, $9^1$s, $9^2$s | The eastern slope of hill '336' | 48.29 | 42.00 | 250 |
| 10 | 10e | Vicinity of Novo-Maka village | 48.32 | 41.75 | 220 |
| 11 | 11e | Vicinity of Novo-Usur village | 48.34 | 41.69 | 320 |
| 12 | 12e | Vicinity of Novo-Ganzah village | 48.35 | 41.63 | 370 |
| | | **Azerbaijan** | | | |
| 13 | 13s | To the south from Hilmilly village | 48.88 | 40.64 | 1000 |
| 14 | 14s | To the south from Kalva village | 48.50 | 40.71 | 760 |
| 15 | 15s | Vicinity of Tirdjan village | 48.34 | 40.76 | 760 |
| 16 | 16s | Dzhebrail district, vicinity of Garar village | 47.01 | 39.42 | 700 |
| 17 | 17s | Vicinity of Zangelan | 46.66 | 39.12 | 490 |
| | | **Armenia** | | | |
| 18 | 18e | Azizlekov – Sisian zoad, near the turn to Dzhezmuk | 45.56 | 39.69 | 1400 |
| 19 | 19e | Vicinity of Noravank village | 45.32 | 39.77 | 1400 |
| | | **Nakhitshevan** | | | |
| 20 | 20e | To the north-west from Nakhitshevan city | 45.24 | 39.28 | 980 |

[1]Given in decimals.

stained as in Harris & Hopkinson (1976) and SKDH was stained as in Neuman & Hart (1983).

ACPH was visualized with 40 mL 0.1 Tris-HCl buffer, pH 5.8, containing 40 mg of 1-naphthyl phosphate and 40 mg of Fast Garnet GBC salt. Prior to staining, the gel was soaked for 1 h at 4°C in 0.2 M Tris-HCl buffer, pH 5.8. Though soaking decreases the total number of ACPH bands scorable, it provides a high intensity of ACPH1, which is of major interest.

Because the character used by Eig (1929) for the separation of *Ae. squarrosa* into the subspecies is *de facto* a quantitative trait, a subspecies index (SI) reflecting the *Ae. squarrosa* spike constitution was worked out. SI was calculated as the ratio of the width of a spikelet glume to the width of the upper part of a rachis segment of the same spikelet. The spikelet from the very middle of the spike was taken for the measurement.

The SIGAMD statistical package was used for conducting principal component analysis, and the author's program written in Pascal was used for conducting multiple correspondence analysis (Lebart *et al*., 1984).

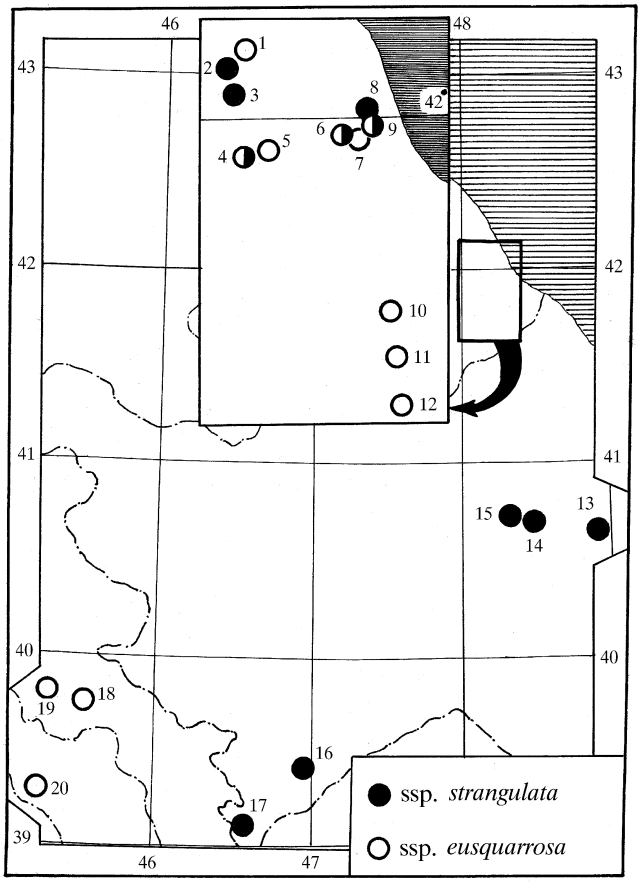


**Fig. 1** Geographical distribution of sampling localities of *Aegilops squarrosa.*

## Results and discussion

Multiple loci in an enzyme system are numbered starting with the most anodal zone. Superscripts on the alleles indicate the relative migration distance on the gel, taking the most frequently found allele as 100 (Table 2). No heterozygotes were found in this study.

EP (endopeptidase) showed on zymograms as two major bands usually supplemented with a third faint band. A preliminary genetic study revealed that different EP zymogram patterns were inherited as allelic variants of a single *Ep* locus. Superscripts on *Ep* alleles are given as double numbers, the first number corresponding to the faster EP band.

### *Variation among locations and populations*

In locations 4, 6 and 9 *Ae. squarrosa* was approximately equally represented by the two subspecies. Allozyme data reveal that *Ae. squarrosa* ssp. *strangulata* in location 9 is represented by a mixture of two populations, evidently owing to some recent migration event. The population $9^1$s, as will be shown below, has been isolated since ancient times. The migration event has taken place in very recent years, because no genetic exchange between $9^1$s and $9^2$s was indicated (Table 2). It was probably the construction of a serpentine road which caused the increase in migration between *Ae. squarrosa* populations located on the slope of hill '336'.

According to Zhukovsky (1928) and Eig (1929), the primary species of the genus *Aegilops* spread out at the end of the Tertiary period. The ancient geographical expansion of *Ae. squarrosa* is reflected in the allozyme spatial distribution patterns. The locations where rare alleles are found are scattered sporadically throughout the species' range (Dudnikov & Goncharov, 1993). High frequency of an unusual allele in a population is a rare event, although independent events of this kind were found, suggesting that *Ae. squarrosa* has been occupying its range for a long time. For example, the $Aco2^{90}$ allele was first found in two neighbouring populations of ssp. *strangulata* in northern Iran and also about 1700 km to the east in a population of ssp. *eusquarrosa* from Uzbekistan (Dudnikov & Goncharov, 1993). Examples of the separate origin of rare alleles also occur in this study. These are: $Ep^{99-100}$ (populations 7e–13s, 14s), $Est5^{66}$ (6e–12e)

and *Mdh2*$^{90}$ (8s, 9$^{1}$s–16s). The *Got3*$^{125}$ allele, which was previously found only in populations from south-eastern Azerbaijan (Jaaska, 1981; Dudnikov & Goncharov, 1993), was found in population 2s, one of the most northern Dagestan populations. The same rare alleles were also found in neighbouring populations (*Ep*$^{99–100}$ in populations 13s, 14s; *Ep*$^{100–102}$ in 3s, 4s; *Mdh2*$^{90}$ in 8s, 9$^{1}$s; *Est1*$^{97}$ in 19e, 20e) indicating some migration between the populations or the origin of neighbouring populations from the same ancestral population (Fig. 1, Table 2).

This study reveals that sspp. *eusquarrosa* and *strangulata* differ distinctly in their ecology, morphology and allozyme variation. Ssp. *strangulata* is found in more moist, higher-located habitats (Fig. 2). Heading in ssp. *strangulata* begins about two weeks later than in ssp. *eusquarrosa.* There is also a clear-cut distinction in SI values between the populations of the two subspecies, with population 4e being the only exception (Fig. 3). Finally, differentiation is clearly demonstrated by the first principal component in a principal component analysis of allozyme variation (Fig. 4).

Data on genetic variation at the 27 enzyme loci studied (Table 2) were used to estimate the relative magnitude of genetic differentiation among populations of *Ae. squarrosa.* Nei's coefficient of gene differentiation $G_{ST}$ was 0.67 for ssp. *eusquarrosa* and 0.64 for ssp. *strangulata* populations.

Although *Ae. squarrosa* is a self-pollinating species (Berlyand-Kozhevnikov & Boguslavsky, 1979), examples of occasional cross-pollination have been found in VIR (Vavilov All-Russian Institute of Plant Industry, St.-Petersburg) accessions (Dudnikov, unpublished data). Evidently, in natural populations, where *Ae. squarrosa* grows sparsely, cross-pollination takes place more rarely than in VIR sown plots used for regenerating germ-plasm material.

**Table 2** Allele frequencies at 11 polymorphic loci in populations of *Aegilops squarrosa*

| | Ssp. *eusquarrosa* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | 1e | 4e | 5e | 6e | 7e | 9e | 10e | 11e | 12e | 18e | 19e | 20e |
| *N*: | 16 | 16 | 16 | 16 | 22 | 9 | 16 | 16 | 16 | 13 | 11 | 15 |
| *Acph1*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Acph1*$^{95}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ep*$^{100–102}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ep*$^{100–100}$ | 0.31 | 0 | 0 | 0.50 | 0.59 | 1.00 | 0.13 | 0.94 | 0.63 | 1.00 | 0.09 | 0 |
| *Ep*$^{99–100}$ | 0 | 0 | 0 | 0 | 0.32 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ep*$^{97–98}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ep*$^{97–96}$ | 0.06 | 0 | 1.00 | 0 | 0.09 | 0 | 0 | 0.06 | 0 | 0 | 0.09 | 1.00 |
| *Ep*$^{97–94}$ | 0.63 | 1.00 | 0 | 0.50 | 0 | 0 | 0.87 | 0 | 0.37 | 0 | 0.82 | 0 |
| *Est1*$^{103}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Est1*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.91 | 0.93 |
| *Est1*$^{97}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.09 | 0.07 |
| *Est2*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 0.86 | 1.00 | 1.00 | 1.00 | 1.00 | 0.15 | 1.00 | 1.00 |
| *Est2*$^{null}$ | 0 | 0 | 0 | 0 | 0.14 | 0 | 0 | 0 | 0 | 0.85 | 0 | 0 |
| *Est2*$^{84}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Est5*$^{170}$ | 0.56 | 0 | 0 | 0 | 0 | 0 | 0.13 | 0 | 0 | 0 | 0.91 | 0.80 |
| *Est5*$^{100}$ | 0.44 | 1.00 | 1.00 | 0.87 | 1.00 | 1.00 | 0.87 | 1.00 | 0.94 | 1.00 | 0.09 | 0.20 |
| *Est5*$^{66}$ | 0 | 0 | 0 | 0.13 | 0 | 0 | 0 | 0 | 0.06 | 0 | 0 | 0 |
| *Got1*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Got1*$^{95}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Got2*$^{105}$ | 0.13 | 0 | 1.00 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Got2*$^{100}$ | 0.87 | 1.00 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Got3*$^{125}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Got3*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Lap*$^{107}$ | 0.06 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lap*$^{100}$ | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Mdh2*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Mdh2*$^{90}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Nadhd2*$^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 0.91 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| *Nadhd2*$^{92}$ | 0 | 0 | 0 | 0 | 0.09 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 2** *Continued*

| | Ssp. *strangulata* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Population | 2s | 3s | 4s | 6s | 8s | $9^1$s | $9^2$s | 13s | 14s | 15s | 16s | 17s |
| *N*: | 21 | 16 | 18 | 16 | 16 | 9 | 3 | 16 | 16 | 16 | 16 | 12 |
| $Acph1^{100}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Acph1^{95}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Ep^{100-102}$ | 0 | 0.06 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Ep^{100-100}$ | 1.00 | 0.94 | 0.66 | 1.00 | 1.00 | 0 | 1.00 | 0 | 0.50 | 0.25 | 0.19 | 0.25 |
| $Ep^{99-100}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.87 | 0.44 | 0 | 0 | 0 |
| $Ep^{97-98}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.42 |
| $Ep^{97-96}$ | 0 | 0 | 0 | 0 | 0 | 1.00 | 0 | 0 | 0 | 0 | 0.81 | 0.33 |
| $Ep^{97-94}$ | 0 | 0 | 0.17 | 0 | 0 | 0 | 0 | 0.13 | 0.06 | 0.75 | 0 | 0 |
| $Est1^{103}$ | 0 | 0 | 0 | 0 | 0 | 1.00 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Est1^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Est1^{97}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Est2^{100}$ | 0.48 | 0.50 | 0.28 | 0.19 | 1.00 | 0 | 1.00 | 0 | 0.19 | 0.13 | 0.19 | 0.25 |
| $Est2^{null}$ | 0 | 0 | 0 | 0 | 0 | 1.00 | 0 | 1.00 | 0.62 | 0.68 | 0.62 | 0 |
| $Est2^{84}$ | 0.52 | 0.50 | 0.72 | 0.81 | 0 | 0 | 0 | 0 | 0.19 | 0.19 | 0.19 | 0.75 |
| $Est5^{170}$ | 1.00 | 1.00 | 0.83 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0.87 | 1.00 | 1.00 | 1.00 |
| $Est5^{100}$ | 0 | 0 | 0.17 | 0 | 0 | 0 | 0 | 0 | 0.13 | 0 | 0 | 0 |
| $Est5^{66}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Got1^{100}$ | 0.71 | 0.87 | 0.94 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 0.37 | 0.63 | 0.56 | 1.00 |
| $Got1^{95}$ | 0.29 | 0.13 | 0.06 | 0.06 | 0 | 0 | 0 | 0 | 0.63 | 0.37 | 0.44 | 0 |
| $Got2^{105}$ | 1.00 | 1.00 | 0.89 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Got2^{100}$ | 0 | 0 | 0.11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Got3^{125}$ | 0.14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Got3^{100}$ | 0.86 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Lap^{107}$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $Lap^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Mdh2^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 0.94 | 1.00 |
| $Mdh2^{90}$ | 0 | 0 | 0 | 0 | 1.00 | 1.00 | 0 | 0 | 0 | 0 | 0.06 | 0 |
| $Nadhd2^{100}$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $Nadhd2^{92}$ | 0 | 0 | 0 | 0 | 0 | 1.00 | 0 | 0 | 0 | 0 | 0 | 0 |

*N*, number of individuals sampled per population.

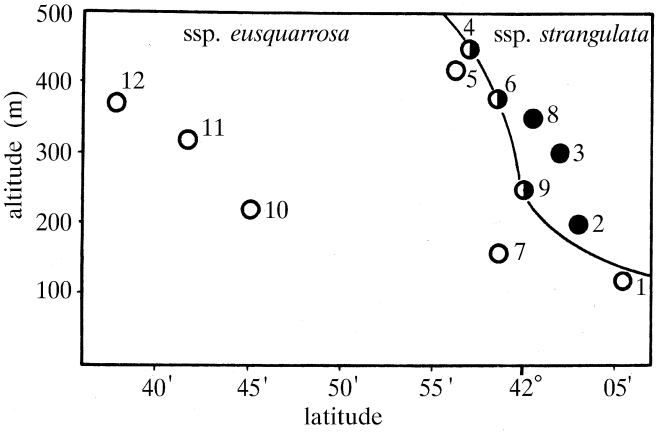


**Fig. 2** The 12 Dagestan primary habitats of *Aegilops squarrosa*.

As expected from the *Ae. squarrosa* mating system and apparently low $N_e$ values, linkage disequilibrium is usually found, although in some populations (1e, 16s, 15s) deviation from gametic phase equilibrium is not significant (Fig. 5).

Despite sharing the same habitat, populations 6s and 6e display no signs of introgression (Table 2, Fig. 3). In contrast, for populations 4s and 4e introgression between the two subspecies is quite obvious. Population 4e stands out from all the other ssp. *eusquarrosa* populations in having a distinct displacement of the SI values towards the moniliform type of spike (Fig. 3). Allozyme variation in *Ae. squarrosa* populations from location 4 also reveals genetic exchange between the two subspecies (Fig. 6).

Although genetic exchange between sspp. *strangulata* and *eusquarrosa* was not found in location 9

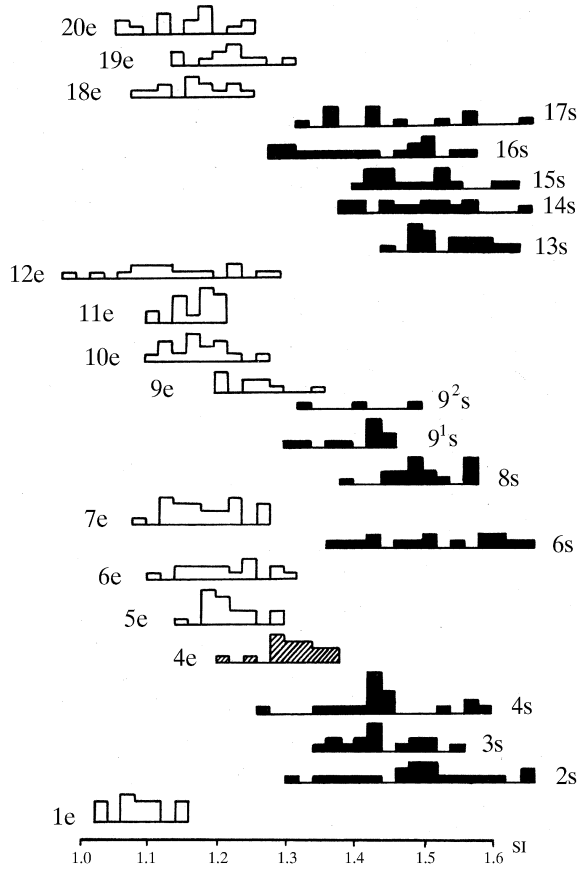


**Fig. 3** Distributions of subspecies index (SI) values in *Aegilops squarrosa* populations.

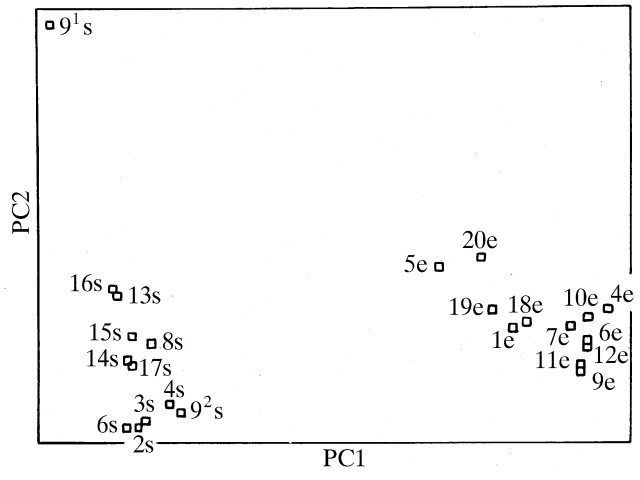


**Fig. 4** Graph of the first two axes from a principal components analysis based on allele frequencies of *Aegilops squarrosa* populations analysed.

directly, the spatial distributions of the alleles $Nadhd2^{92}$ and $Ep^{97-96}$ provide some evidence that introgression has taken place somewhere in the vicinity of hill '336'. Both $Nadhd2^{92}$ and $Ep^{97-96}$ attain a frequency of 100 per cent in the $9^1$s population of ssp. *strangulata*. The neighbouring 7e population of ssp. *eusquarrosa* is the only other population in this and the previous study (Dudnikov & Goncharov, 1993) where the $Nadhd2^{92}$ allele was found. It was also the only other population among those in the vicinity of hill '336' (6s, 6e, 7e, 8s, $9^1$s, $9^2$s, 9e) where the $Ep^{97-96}$ allele was found (Table 2). In population 7e the plants which had the $Nadhd2^{92}$ allele were the only ones which had the $Ep^{97-96}$ allele (Fig. 5).

*Ep*, *Est2* and *Got1* are commonly polymorphic in ssp. *strangulata*, so that the spatial distribution of *Ep* allele frequencies can be compared with those of *Est2* and *Got1*. When *Ep* is considered, $9^1$s stands apart from all the other ssp. *strangulata* populations because it is the only one in which the allele $Ep^{97-96}$ had a frequency of 100 per cent. For the other 11 populations, it can be seen that the allozyme variation patterns correspond in general with the geographical distances between the populations (Fig. 7). The spatial distribution patterns of the *Est2* and *Got1* allele frequencies are quite different from those of *Ep*, displaying no correlation with the geographical distance between populations. Geographically distant populations can be remarkably similar, whereas neighbouring populations can differ greatly (Fig. 8). In general, the eastern Dagestan populations 8s, $9^1$s and $9^2$s and the most eastern among the Azerbaijan group of populations (13s) all lack both the $Est2^{84}$ and $Got1^{95}$ alleles. When the other eight populations of ssp. *strangulata* are considered, the $Est2^{84}$ frequency correlates negatively with the frequency of $Got1^{95}$ ($r = -0.91$, $P < 0.002$).

### *Differences among loci*

The 27 enzyme loci studied fall into four reasonably distinct groups.

**1** The monomorphic loci: *Aco1*, *Aco2*, *Acph4*, *Ald*, *Cat1*, *Cat2*, *Est3*, *Est4*, *Gapd*, *Gdh*, *Gp*, *Gpi*, *Mdh1*, *Nadhd1*, *Pgm*, *Skdh*.

**2** *Est1*, *Got3*, *Lap*, *Mdh2*, *Nadhd2*. In most populations each of these loci is represented only by its common allele. If a rare allele was found, it either had a low frequency or was fixed.

**3** *Ep*. This is the highly polymorphic locus with variation patterns similar in both the subspecies.

**4** *Acph1*, *Est2*, *Est5*, *Got1*, *Got2*. Their allozyme variation patterns differ greatly between the two

subspecies. These were the five loci in which allozyme variation separated *Ae. squarrosa* into ssp. *eusquarrosa* and ssp. *strangulata* along the first principal component in Fig. 4.

Lewontin (1985) criticized the rather common approach in population genetics studies of treating different loci as a homogeneous collection. It was emphasized that the correct classification of different loci in terms of the general forces acting on them is desirable. In the case of *Ae. squarrosa,* heterogeneity among the enzyme loci studied is apparent.

The most probable reason for the monomorphism of the enzyme loci in the first group is purifying

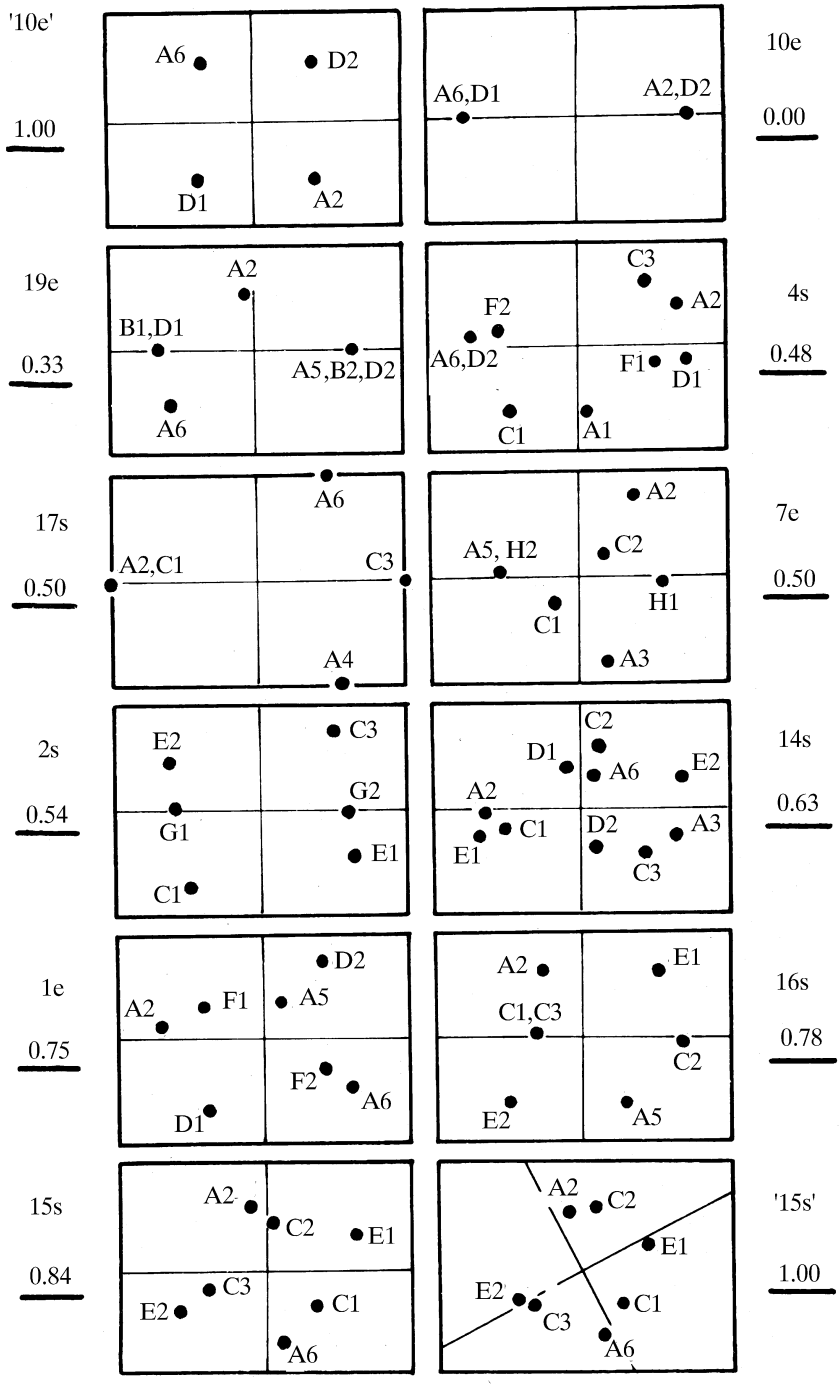


**Fig. 5** Graphs of the first two axes from multiple correspondence analysis. For each population of *Aegilops squarrosa*, specimens were taken as variables and genetic markers as objects. A1, $Ep^{100-102}$; A2, $Ep^{100-100}$; A3, $Ep^{99-100}$; A4, $Ep^{97-98}$; A5, $Ep^{97-96}$; A6, $Ep^{97-94}$; B1, $Est1^{100}$; B2, $Est1^{97}$; C1, $Est2^{100}$; C2, $Est2^{null}$; C3, $Est2^{84}$; D1, $Est5^{170}$; D2, $Est5^{100}$; E1, $Got1^{100}$; E2, $Got1^{95}$; F1, $Got2^{105}$; F2, $Got2^{100}$; G1, $Got3^{125}$; G2, $Got3^{100}$; H1, $Nadhd2^{100}$; H2, $Nadhd2^{92}$. The same location of alleles of different loci in the multidimensional space formed by the principal axes indicates 100 per cent association of the alleles in the population. The same projection of C1 and C3 in a plane formed by the first two principal axes (16s population) indicates that these *Est2* alleles display entirely the same association patterns with all the other genetic markers in the population. The ratio $\lambda_2/\lambda_1$ (the trivial eigenvalue $\lambda_0 = 1$ is not considered) is used as a general measure of the extent of linkage disequilibrium in a population. It can vary from 0 to 1, the latter extreme value corresponding to the case of complete gametic phase equilibrium. $\lambda_2/\lambda_1$-values for each population are given as underlined numbers. The '10e' and '15s' graphs give examples of how the patterns at the 10e and 15s populations would look in the case of complete linkage equilibrium.

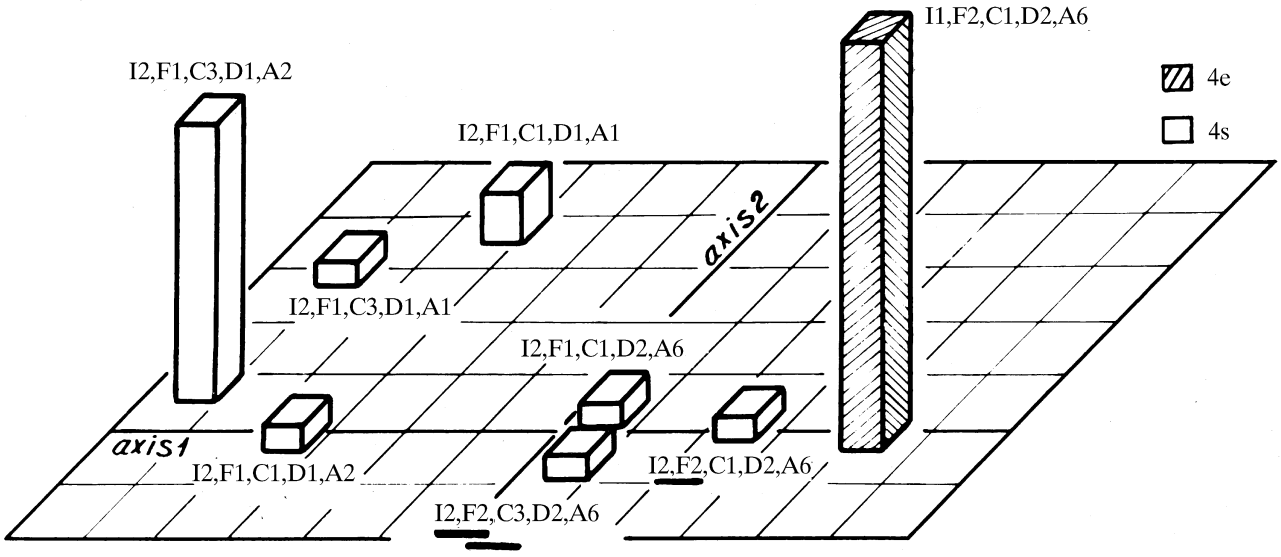


**Fig. 6** The first two axes from multiple correspondence analysis. Genetic markers were taken as variables and the 34 specimens of both *Aegilops squarrosa* ssp. *eusquarrosa* and ssp. *strangulata* from location 4 were taken as objects. The number of specimens of each genotype found is reflected by the height of the column. Allele designations are listed in Fig. 5, except I1, $Acph1^{100}$; I2, $Acph1^{95}$. In the genotype descriptions only one allele of each locus is indicated because no heterozygotes were found. The unusual *Ae. squarrosa* gene combinations are underlined.

selection against alternative allelic variants. Low mutation rates of the loci are unlikely to be the cause, because the same locus in another environment, or its homologous locus in a closely related species, can be highly polymorphic. For example, *Cat2*, which is monomorphic in both *Ae. squarrosa* subspecies in the Transcaucasian region, is represented by three approximately equally common alleles in the eastern part of the *Ae. squarrosa* range inhabited by ssp. *eusquarrosa* (Dudnikov & Goncharov, 1993). The *Gdh*, *Gp* and *Gpi* loci from the first group were found to be polymorphic in each of the five species of the *Sitopsis* group of *Aegilops* studied electrophoretically by Mendlinger & Zohary (1995).

Rare alleles of the loci from the second group are likely to be slightly deleterious. These alleles never attain high frequencies, except for occasionally being fixed by genetic drift on account of the small size of the *Ae. squarrosa* populations (Table 2). If some of the *Ae. squarrosa* populations remained isolated and free from immigration over a long period of time, they may have become a 'trap' for rare alleles because of fixation of slightly deleterious alleles. It seems reasonable to suggest that population $9^1$s or its ancestral population is an example of this (Table 2, Fig. 4). Another example is the accession VIR-1954, Iran, which has fixation of the alleles $Aco2^{110}$ and $Nadhd1^{88}$ which have not been found anywhere else in *Ae. squarrosa* (Dudnikov & Goncharov, 1993).

Obviously, the dividing line between the first and the second group of loci is to some extent arbitrary because it depends upon the amount of plant material studied. If the data on VIR *Ae. squarrosa* accessions studied earlier (Dudnikov & Goncharov, 1993) are considered, the *Aco2*, *Acph4*, *Mdh1*, *Nadhd1* and *Pgm* loci have to be assigned to the second group.

The loci from the remaining two groups are essentially polymorphic in *Ae. squarrosa* if the species is considered as a whole. Stochastic processes and/or natural selection may be the reasons for this polymorphism. In the case of *Ae. squarrosa*, neutral genes and genes under natural selection could be expected to display distinctly different variation patterns.

### *Evolutionary considerations*

*Aegilops squarrosa* has inhabited its range since the end of the Tertiary period (Zhukovsky, 1928; Eig, 1929) so that the frequency distribution of any allele studied among local populations of any of the subspecies can be expected to be in equilibrium. Apparently, the population genetic structure of the two subspecies is not appreciably different, which is also reflected by the similar $G_{ST}$ values in both

subspecies. Thus, for a neutral gene, the distributions are expected to be similar in both the subspecies. Genetic exchange between the subspecies, which was found to be rather common, must also contribute to this similarity. By contrast, in the case of a gene under natural selection, the gene frequency distributions can differ widely in the two subspecies as a result of distinct differences in their ecology and genetic constitution.

It should be noted that, because *Ae. squarrosa* inhabits mountainous or hilly regions, geographically close habitats can be ecologically quite different, and conversely, habitats, which are geographically distant from one another, can be very similar in their environmental conditions. So, allozyme variation, if under natural selection, can be expected to display, in general, no correlation with geographical distances between the populations. Allele frequencies at different loci may correlate with each other if they are under the influence of the same environmental factors. On the other hand, in the case of neutrality, similarity in allozyme variation patterns between geographically close populations may be expected as a result of migration or origins from the same ancestral population.

The fourth group of loci appear to be subjected to natural selection. Despite genetic exchange between

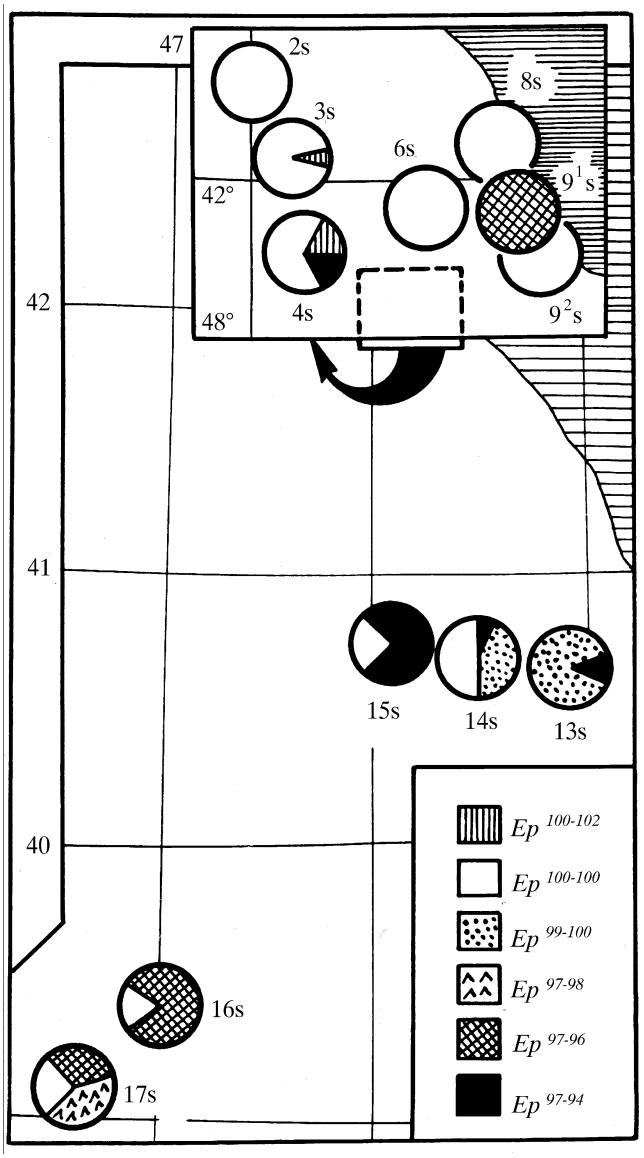


**Fig. 7** Spatial distribution of *Ep* allele frequencies in *Aegilops squarrosa* ssp. *strangulata* populations.

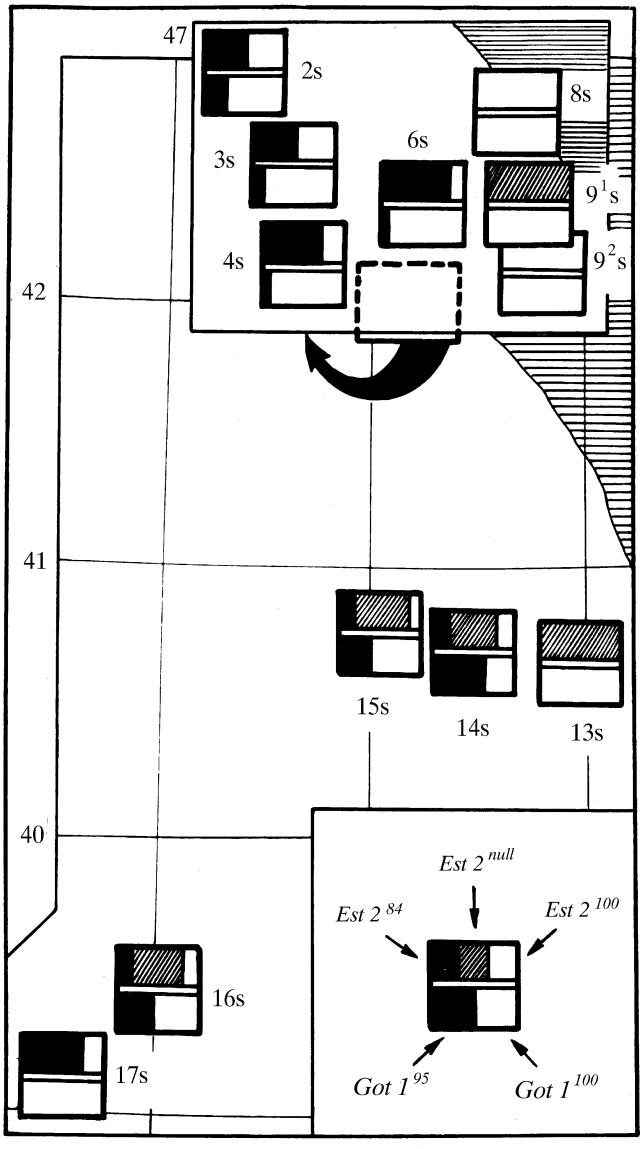


**Fig. 8** Spatial distribution of *Est2* and *Got1* allele frequencies in *Aegilops squarrosa* ssp. *strangulata* populations.

the two subspecies, $Acph1^{95}$ is the only *Acph1* allele that was found in ssp. *strangulata*, whereas ssp. *eusquarrosa* has exclusively the $Acph1^{100}$ allele. $Got2^{105}$, which is the only or predominant *Got2* allele in ssp. *strangulata* populations, displays in ssp. *eusquarrosa* the type of frequency distribution that is characteristic of the slightly deleterious alleles for loci in the second group. In ssp. *strangulata*, $Got1^{95}$ and $Est2^{84}$ display a distinctly asymmetrical, approximately hyperbolical type of frequency distribution, which could indicate natural selection (Wright, 1931, 1937). In ssp. *eusquarrosa* these alleles are completely absent, suggesting strong purifying selection (Table 2). The $Got1^{95}$ frequency correlates negatively with the frequency of $Est2^{84}$ in those populations of ssp. *strangulata* which have at least one of these alleles. The spatial distributions of the *Got1* and *Est2* allele frequencies (Fig. 8) are consistent with what is expected in the case of natural selection.

The neutrality model is the most likely explanation for the *Ep* variation patterns in *Ae. squarrosa.* Environmental factors seem to have no influence on *Ep* variation. All the five enzyme loci (*Acph1*, *Cat2*, *Est2*, *Est5* and *Got2*) which in the previous study (Dudnikov & Goncharov, 1993) were found to be essentially polymorphic in *Ae. squarrosa* in general, were polymorphic in some parts of the vast *Ae. squarrosa* range but monomorphic in others. *Ep* remains polymorphic through all the *Ae. squarrosa* distribution from Georgia to Kirgizia (Dudnikov, unpublished data). *Ep* variation patterns are similar in ssp. *eusquarrosa* and ssp. *strangulata* (Table 2), despite the differences in the genetic constitutions and ecological preferences of the subspecies. $Ep^{100-100}$, the most common *Ep* allele, displays an approximately regularly spaced frequency distribution in unfixed factors, which suggests neutrality (Wright, 1931, 1937). *Ep* displays spatial variation patterns that differ greatly from those of the loci under selection (Figs 7 and 8). As expected, there is similarity between the geographically close populations in their *Ep* variation patterns. Population $9^1$s is the only marked exception, which is consistent with the evidence obtained from the variation patterns of the slightly deleterious alleles, that this population has been isolated for a long period of time.

According to Jaaska (1981), subspecies *strangulata* and *eusquarrosa* differentiated at the very beginning of the appearance of *Ae. squarrosa* as a species. It was an adaptive process, and five out of the 27 enzyme loci studied appear to have been involved in it. Despite the genetic exchange between them, ssp. *eusquarrosa* and ssp. *strangulata* remain as two distinct subspecies, suggesting that intermediate genotypes are disadvantageous.

Future intraspecies differentiation of *Ae. squarrosa* could finally lead to the origin of a new species through nonadaptive differentiation of small isolated populations. The populations $9^1$s (Fig. 4) and VIR-1954, Iran, may be examples of such differentiation.

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