by the conserved region vaccine and introduced by this delivery system. It is clear that type-specific antibodies to the M molecule are necessary to protect the host once the streptococcus has initiated infection (*7, 8*). However, our studies reveal that an immune response to the non–type-specific regions will block those events necessary for streptococcal colonization of the mucosal surface in this model system. Although the conserved region used in these studies exhibits >80% sequence identity with known M molecules (*24*), the extent of cross-protection provided by this segment among the known M serotypes remains to be determined.

Whereas the use of VV-based vaccines is not likely to be approved for the control of disease in the near future because of medical concerns regarding safety, the research reported here for the streptococcal M protein provides an example of how such vectors may be used to define the contribution of individual epitopes in the induction of a protective immune response. This approach may be applicable for protection against other pathogenic organisms for which no vaccines now exist, because of problems ascribed to either serotype diversity or antigenic variability of major virulence determinants.

Note added in proof: Experiments were designed to determine if immunization with VV:M6′ is also able to protect mice against colonization after challenge with heterologous streptococcal serotypes. Among 12 mice immunized with VV:M6′ and challenged both intranasally and orally with M14 streptococci (strain T14/46), 17 to 25% exhibited positive throat cultures over a 6-day period while 50 to 70% of 10 animals immunized with either wild-type VV or no virus were colonized by the M14 organisms. By day 6 post-challenge, 20% of the control animals and none of the VV:M6′ immunized animals had died. Although these experiments need to be repeated with this and other streptococcal serotypes, they suggest that immunization with the conserved region of the M6 molecule will significantly reduce colonization by both homologous and heterologous streptococcal serotypes.

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13. The pVV:M6Δ plasmid was cut with Fok I (cleaves at position 751 of the M6 sequence) and Cla I (cleaves at the 3′ end of the insert). The fragment corresponding to the 3′ half of the M6 gene was isolated, the recessed ends filled in with the Klenow fragment of DNA polymerase I, and the blunt-ended fragment cloned into the Sma I site of M13mp19 replicative form DNA. A recombinant phage containing the insert in the correct orientation was identified by hybridization with a strand-specific oligonucleotide probe. Single-stranded DNA was prepared and used as a substrate for oligonucleotide-directed site-specific mutagenesis designed to introduce a G between positions 769 and 770 of the insert. This alteration, which was verified by Sanger dideoxynucleotide sequencing procedures, resulted in the Lys^{209} codon of the M6 open reading frame being converted to an in-frame AGT codon. The mutagenized insert (M6′) was excised from the phage DNA with Eco RI and Bam HI, which cut at the 5′ and 3′ ends, respectively, of the M6′ insert. The recessed ends were blunted with Klenow, and the fragment was blunt-end ligated into the Bam HI site of the pVV3 insertion plasmid, which was also filled in with Klenow. Restriction map and nucleotide sequence procedures were used to select a recombinant plasmid containing the M6′ insert in the correct orientation with regard to the VV 7.5-kD promotor element. This pVV3:M6′ recombination plasmid was used to introduce the chimeric gene into the VV genome by using standard marker transfer technology [C. M. Rice, C. A. Franke, J. H. Strauss, D. E. Hruby, *J. Virol.* **56**, 227 (1985)]. The recombinant virus (VV:M6′) was grown, purified, and then carefully analyzed to verify that the M6′ insert was present, intact, and actively transcribed in virus-infected cells. The genotype of VV:M6′ was apparently quite stable as no deletions or rearrangement of the M protein sequences were evident after multiple passage of the recombinant VV through tissue culture cells.
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25. We are grateful to T. Guinta for her assistance with the animals, M. Windels for technical assistance, and E. C. Gotschlich and D. Bessen for helpful advice during these studies. Supported in part by NIH grants AI-11822 (V.A.F.) and AI-26281 and Research Career Development Award AI-00666 (D.E.H.).

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Plant Hybrid Zones as Sinks for Pests

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An 8-year study of how aphids are distributed and survive on hybrid and pure host populations showed that the more susceptible hybrid trees acted as pest sinks supporting most of the aphid population. At least 85 to 100 percent of the aphid population was concentrated on less than 3 percent of the host population, with the center of a pest's distribution being the hybrid zone of its host. The concentration of aphids on such a small segment of the host population suggested that susceptible plants not only acted as sinks in ecological time, but may also have prevented aphids from adapting to the more numerous resistant hosts in evolutionary time. This has important implications for the potential management of pest evolution in agriculture and in understanding natural pest distributions.

ALTHOUGH RARELY EXAMINED, THE unparalleled genetic variation that can arise when two plant species hybridize and introgress represents an opportunity to examine how insects and pathogens respond to genetically scrambled hosts (*1*). Since hybridization is known to affect resistance to pests and parasites (*2*), it is of interest to determine how altered host resistance in natural hybrid zones might affect the evolution of plant-pest interactions. Here I examine how a plant parasite, the gall-producing aphid, *Pemphigus betae*, is ecologically and perhaps evolutionarily tied to natural hybrid cottonwoods, *Populus* sp. Most of the study was concentrated along approximately 500 km of the Weber River in northern Utah where Fremont cottonwood, *Populus fremontii*, occupies the lower elevations of riparian habitat and narrowleaf cottonwood, *P. angustifolia*, occupies the upper elevations. A 13-km zone of overlap exists at their common boundary where both species interbreed to produce a hybrid swarm.

The extent of hybridization is demon-

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strated by morphological, biochemical, and genetic analyses. For example, no overlap in leaf morphologies of trees from pure stands of each species was observed (Fig. 1, A and C); however, where both species occur, a continuum of leaf morphologies was apparent (Fig. 1B). Analyses of phenolics and experimental crosses also confirmed hybrid status (*3*). Furthermore, RFLP (restriction fragment length polymorphism) analyses showed that successful hybrid matings occurred only between hybrids and narrowleaf cottonwood. Thus, hybrids do not breed true, introgression is directional, and hybrids cannot be considered a separate species (*3*).

Because of 75-fold differences in survival on individual trees, the selection of a quality host represents an important stage in the aphid life cycle (*4*). In early spring a gall-forming female (the stem mother) attempts to initiate a gall on a developing leaf and, if successful, parthenogenetically produces up to 300 progeny. Because stem mothers that die during gall initiation leave a scar as evidence of their failed attempt, the resistance of the host tree can be quantified as the survival rate of these colonizers (*4*). For example, of the 1074 gall attempts recorded for one tree, 75% survived, whereas, of the 95 attempts recorded for a nearby tree, none survived. In response to this variation, colonizing aphids discriminate between trees within a site and selectively colonize the most susceptible trees year after year (*4*).

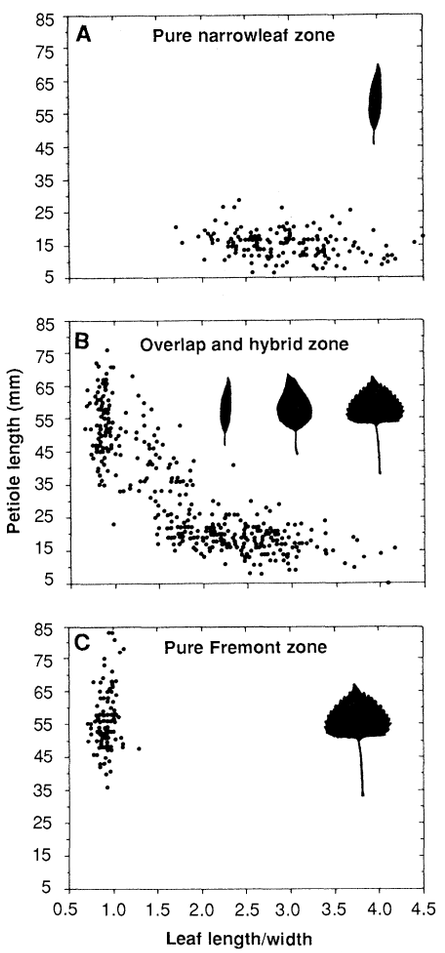

Fig. 1. The distribution of pure and hybrid cottonwoods along the Weber River is shown from leaf traits. (**A** and **C**) In comparisons of 152 trees in pure stands of narrowleaf cottonwood with 104 trees from pure stands of Fremont cottonwood, no overlap in leaf traits was observed. (**B**) However, analyses of 374 trees in the 13-km zone where both species are found show a continuum of leaf traits indicating the presence of a hybrid swarm. Silhouettes of leaves characteristic of each zone are shown.

Clonal variation in resistance is genetically based and related to the level of hybridization. A random sample of 16 mature trees from the hybrid zone with aphid survival rates ranging from 0 to 75% were vegetatively cloned and grown for 4 years in a common garden free of aphids. The survival rates of 3198 aphids transferred onto these derivative clones were then compared with the survival rates of 8458 aphids that had naturally colonized the parental trees. The bivariate distribution so generated shows a strong relation between the resistance of a clone and the parental tree from which it was derived ($r = 0.90$, $P < 0.001$). Furthermore, regression of clones on parents yields a slope near 1 [$b = 0.98 \pm 0.127$ (1 SE)], indicating that the resistance traits of derivative clones do not differ significantly from their parents. Although nongenetic factors could be involved, heritability studies of clonal traits in cottonwoods and other plants demonstrate a strong genetic component to such traits (*5*). This conclusion is further supported by RFLP data showing a significant correlation between susceptibility and the level of backcrossing (*6*).

These results suggest that host resistance might also affect the distribution of aphids over a broad geographic area, with hybrid zones being the most susceptible due to hybrid breakdown. Although several mechanisms may result in hybrid breakdown (*7*), the loss of vigor, resistance, viability, and other ecologically important traits have been associated with hybrid zones and are thought to be important barriers between species (*1*, *2*, *7*). To examine how aphids were distributed relative to the distribution of hybrids and their parental cottonwood species, censuses were conducted for the years 1981 through 1986.

These censuses showed that the center of aphid abundance is the hybrid zone of its host species (Fig. 2). Mean gall densities over the 6-year period in the pure Fremont, hybrid, and pure narrowleaf zones were 0.0 $\pm$ 0.0 (1 SE), 559.4 $\pm$ 119.7, and 4.7 $\pm$ 2.9 galls per 10,000 leaves, respectively. Analyses of variance followed by Newman-Keuls show that in all 6 years, trees in the hybrid zone supported far more aphids than the pure host zones ($P < 0.01$ in all years).

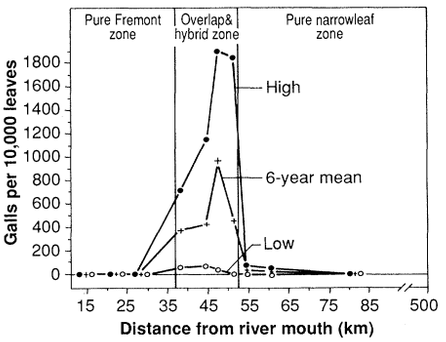

Fig. 2. The center of abundance for the gall aphid, *P. betae*, is the hybrid zone of its host species. Because censuses included both the galls of stem mothers that died trying to establish a gall and those that survived, these censuses represent an accurate estimate of the colonizing population. The distribution of 2776 galls on 610 trees at ten sites was recorded for a 6-year period.

Two additional conclusions can be drawn from these results. First, the total absence of *P. betae* from Fremont cottonwood shows that this species is not used as a host. Furthermore, when stem mothers were experimentally transferred to these trees, they were unable to make galls, and all died. Second, the boundary between high gall densities and very low gall densities is abrupt and occurs over a short distance that closely matches the distribution of hybrid and pure host zones. For example, in progressing from the hybrid to pure narrowleaf zone, in less than 3 km and an elevational gain of 10 m, gall densities dropped from a 6-year mean of 457 to only 18 galls per 10,000 leaves. Thus, even though there is some aphid spillover into the pure narrowleaf zone at the boundary, aphid densities drop sharply. Excluding the boundary site, gall densities throughout the rest of the pure narrowleaf zone are so low as to represent rare events (for example, mean gall densities at two sites well into the pure zone were 9.5 $\pm$ 5.3 and 0.1 $\pm$ 0.0 galls per 10,000 leaves, respectively). Mean aphid density in the hybrid zone was 119 times as great as that in the pure host zone.

The extreme concentration of aphids in the hybrid zone suggests that these trees are more susceptible and thus more attractive as hosts to aphids. Two lines of evidence support this hypothesis. First, the survival rates of stem mothers that naturally attempted gall formation in both zones were examined. Aphid survival on trees in the hybrid swarm was significantly greater than in the pure host zone; of the 1151 galls examined from 61 trees in the hybrid zone, 60.0% of the

stem mothers survived, whereas of the 819 galls examined from 122 trees in the pure zone only 39.2% survived [$\chi^2(1) = 83.196$, $P < 0.001$].

Second, in 1987, when 4000 stem mothers were experimentally transferred onto 100 trees within both zones, the same patterns emerged; trees in the hybrid zone were more susceptible than trees in the pure host zone (Fig. 3). The average aphid survival on trees in the hybrid zone was 60.7% compared to only 38.5% on trees in the pure host zone [$t(98) = 6.861$, $P < 0.001$]. Thus, both natural and experimentally determined survival rates yielded similar results; trees from the hybrid zone are more susceptible to aphid attack.

To test the generality of these findings, another river drainage system was examined in 1988, and identical patterns resulted. Trees in the hybrid zone along the Ogden River were also attacked at a much higher rate than trees in the pure zones (*8*). Furthermore, aphid survival on trees in the hybrid zone was also significantly higher than the survival rate observed in the pure host zone (*8*). For both watersheds studied, alternative hypotheses involving overwintering survival, plant stress, and other factors that might result in the same patterns could not be supported (*9*).

These data not only support the hypothesis that aphid distributions are determined by the distribution of susceptible hosts but that most of the aphid population is derived from very few hosts. Using aphid abundance data, aphid survival rates, and host abundance data for all zones, I estimated the relative importance of the hybrid zone in contributing to future aphid generations (*10*). Although the hybrid zone represents less than 3% of the potential host population in Weber River drainage system, in an average year these trees account for a minimum of 85% of all surviving stem mothers and their progeny. Furthermore, in low-density years when no galls were found in the pure narrowleaf zone, the hybrid host zone gave rise to 100% of the surviving aphids. Thus, when the aphid population is at its lowest, the susceptible trees of the hybrid zone apparently act as a refugium.

The concentration of aphids on the more susceptible trees in the hybrid zone raises the question, "Why have aphids failed so spectacularly on the more numerous parental host species, particularly since aphids and their relatives are well known for their ability to evolve biotypes (*11*)?" Studies of relaxed selection pressures may provide the answer. Numerous experiments with insecticides have shown that when selection is relaxed as when a toxin is no longer used, pests that had evolved resistance to the toxin may subsequently lose their resistance (*12*). In the aphid-cottonwood system presented here, the combination of relaxed selection and high aphid performance on susceptible hybrids may swamp the genetic contribution of the few aphids that are adapted to resistant plants. Under such conditions aphids may have lost or never evolved the ability to successfully attack the more resistant parental host species. If, in addition to concentrating pests and acting as sinks in ecological time, susceptible plants prevent pests from becoming adapted to resistant hosts, they should also be considered evolutionary sinks.

As these and other studies suggest, the potential may exist to use a low percentage of susceptible plants in agricultural systems to manage pest evolution along less harmful pathways. Atsatt and O'Dowd (*13*) argued that the presence of susceptible host species or varieties could lengthen the useful life of resistant hosts by slowing pest evolution. Thus, important genes for resistance may be conserved. Simulation models by Gould for mixing resistant and susceptible varieties of wheat (*14*) also support this logic. Because this study shows that aphids have not adapted to the pure host species even though they should have had ample evolutionary time to do so, it would appear that pest adaptation to the more resistant pure hosts has not only been slowed, but perhaps prevented. In the search for durable resistance in agricultural systems (*15*), in some instances host susceptibility may provide a useful tool in combating the advances of rapidly evolving pests.

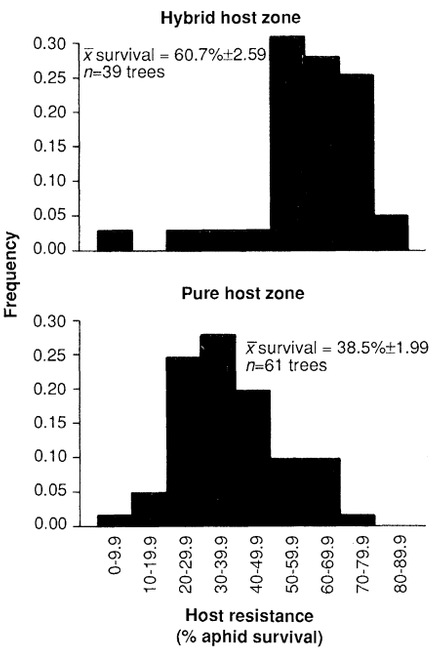

Fig. 3. The average tree in the hybrid host zone is significantly more susceptible to aphid attack than the average tree in the pure narrowleaf host zone [$t(98) = 6.861$, $P < 0.001$; mean ± 1 SE are shown]. Approximately 40 stem mothers were experimentally transferred onto each of 100 trees and their subsequent survival rates recorded. Overwintering eggs were collected from a single source tree to eliminate donor effects. Eggs were refrigerated and, when exposed to ambient temperatures, hatched within a few days. These first instar wingless stem mothers were then transferred to trees in both zones when the buds were beginning to break. Stem mothers were individually transferred to small branches and a sticky barrier was placed at the base of each branch to prevent emigration; survival rates were recorded about 45 days later.

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8. Patterns of distribution and survival for the Ogden River drainage system are identical to those of the Weber River drainage system. For example, gall densities in the hybrid zones were much greater than those in either pure host zones ($\bar{x} = 0.0$ galls per 1000 leaves for 71 trees in the pure Fremont zone; ($\bar{x} = 277.6 \pm 40.1$ galls per 1000 leaves for 68 trees in the hybrid zone; $\bar{x} = 15.3 \pm 4.2$ galls per 1000 leaves for 48 trees in the pure narrowleaf zone; $F(2,184) = 40.0069$, $P < 0.0001$). Although the absence of *P. betae* in the pure Fremont zone prevented an assessment of natural survival, survival rates were significantly higher in the hybrid zone than in the pure narrowleaf zone (63.3% survival of 539 galls examined from 68 trees in hybrid zone compared to 32.7% survival of 104 galls from the 48 trees in pure narrowleaf zone; $\chi^2(1) = 33.522$, $P < 0.001$).
9. Alternative hypotheses that have been experimentally examined include (i) greater overwintering mortality in pure sites; however, eggs that have overwintered in pure zone do just as well as those from the hybrid zone (T. Whitham, unpublished data); (ii) the potential lack of secondary hosts in the pure zone was rejected by N. A. Moran and T. G. Whitham [*Evolution* **42**, 717 (1988)]; (iii) increased water stress in the hybrid zone may make these plants more susceptible; however, increased water stress at lower elevation sites acts to reduce aphid population numbers, making the observed patterns conservative [N. A. Moran and T. G. Whitham, *Ecology* **69**, 1214 (1988)].
10. The relative importance of the hybrid zone to the aphid population in terms of where most surviving aphids are produced was estimated from census abundance data, survival data, and the size of each

zone. For example, I used census data to calculate relative aphid abundance (A) for each zone, (that is, from Fig. 2, stem mothers are 119 times more abundant per kilometer in the hybrid zone than in the narrowleaf zone). In addition, aphid transfer experiments showed that survival rates (S) were different for each zone (that is, from Fig. 3, stem mother survival averaged 60.7% in the hybrid zone compared to 38.5% in the pure narrowleaf zone). Finally, by using maps of the drainage system I estimated the linear size (K) of each riparian zone (that is, 13 km of hybrid and 430 km of pure narrowleaf zone). Thus, the relative contribution of each zone to the aphid population is the product of $A \times S \times K$ (that is, gall abundance per kilometer of habitat × survival × the river kilometers of each habitat).

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Transfer RNA Genes: Landmarks for Integration of Mobile Genetic Elements in *Dictyostelium discoideum*

ROLF MARSCHALEK, THOMAS BRECHNER, ELFI AMON-BÖHM, THEODOR DINGERMANN

In prokaryotes and eukaryotes mobile genetic elements frequently disrupt the highly conservative structures of chromosomes, which are responsible for storage of genetic information. The factors determining the site for integration of such elements are still unknown. Transfer RNA (tRNA) genes are associated in a highly significant manner with different putative mobile genetic elements in the cellular slime mold *Dictyostelium discoideum*. These results suggest that tRNA genes in *D. discoideum*, and probably tRNA genes generally in lower eukaryotes, may function as genomic landmarks for the integration of different transposable elements in a strictly position-specific manner.

THE MAJOR FUNCTION OF TRANSFER RNA genes (tDNA) is to provide genetic information for tRNAs, molecules that act as adaptors for amino acids to ensure the faithful translation of the genetic information into proteins. However, other functions of tRNA genes or tRNA-like genes are known. On the compact mitochondrial genomes of mammals, tRNA genes separate other genes and mark positions for processing (*1*), and tRNA gene-like structures on retroviral genomes act as primers to copy the genetic information of these viruses (*2*). In *Saccharomyces cerevisiae*, tRNA genes are frequently associated with mobile genetic elements (transposons) (*3, 4*). The reason for this tight association is unclear.

We have isolated and cloned 24 genomic

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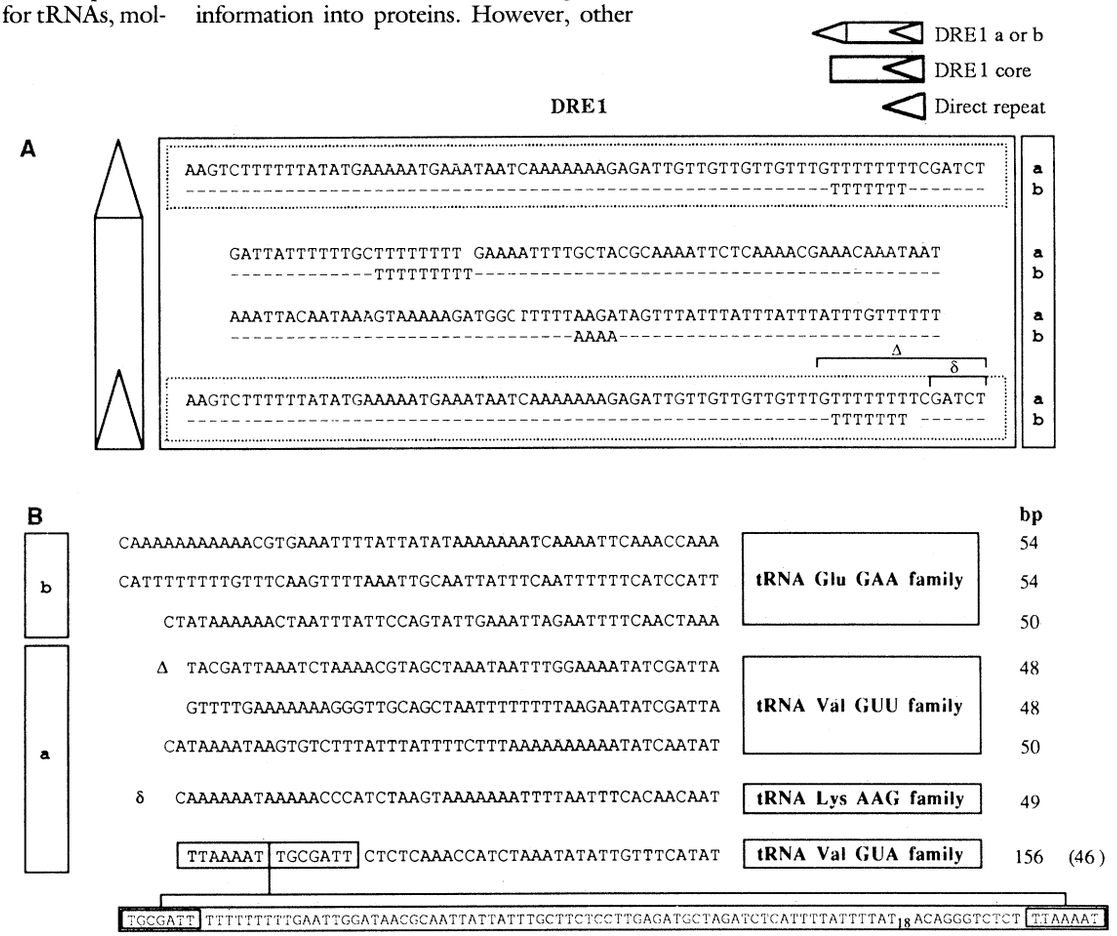

Fig. 1. Nucleotide sequence of DRE1 elements (**A**) and their position relative to different *D. discoideum* tRNA genes (**B**). (A) The nucleotide sequence (written in 5′→3′ direction toward the tRNA genes) of variants a and b of the DRE1 element is shown. They consist of a core element of 199 bp in addition to a direct repeat of nucleotides 1 to 72 in case of DRE1a or 1 to 71 in case of DRE1b. Slightly truncated forms are associated with $tRNA^{Val}(GUU)1$ with nucleotides deleted marked with Δ, and with $tRNA^{Lys}$-(AAG)1 with nucleotides deleted marked with δ. (B) Nucleotides that separate *D. discoideum* tRNA genes and associated DRE1 elements.