The evolutionary history of an invasive species: alligator weed, *Alternanthera philoxeroides*

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Summary

The eco-evolutionary mechanisms of biological invasions are still not thoroughly understood. Alligator weed, *Alternanthera philoxeroides* (Martius) Gisebach (Amaranthaceae), is a plant native to South America and a weed in Australia and other countries. To better understand its success as an invader, we assessed the morphological and cytogenetic variability of 12 Argentine populations and the cytogenetic variability of seven Australian populations. We found differences in leaf shape (width-to-length ratio) and stem architecture in the Argentine populations, in reproduction (sexual with regular meiosis in two Argentine populations vs completely asexual with irregular meiosis and low pollen viability in all other populations) and ploidy level (tetraploids with sexual reproduction and seed production vs hexaploids with or without sexual reproduction). We propose a hypothesis about the mechanism that drove alligator weed to form highly invasive hybrid populations with vegetative reproduction from diploid ancestors, and we consider the implications for plant–herbivore interactions and biological control of this weed.

Keywords: *Alternanthera philoxeroides*, biological invasions, plant–herbivore interactions, hybrids, polyploids.

Introduction

The identification and characterization of the native range and the centre of origin of a weed are crucial in a biological control program. Alligator weed, *Alternanthera philoxeroides* (Martius) Gisebach (Amaranthaceae), is a target of biological control in Australia. Its native range is southern South America (Argentina, Paraguay, Uruguay and Brazil). In Argentina, the genus *Alternanthera* Forsskal includes 27 species, four of them endemic (Pedersen, 1999), indicating that this is probably its natural area of distribution and perhaps its centre of origin. Alligator weed is represented by two known morphological forms in Argentina, *A. philoxeroides* f. *philoxeroides* (Mart.) Griseb. and *A. philoxeroides* f. *angustifolia* Süssenguth, and a third, intermediate form, all of which were recently associated to a complex of hybrids (Sosa *et al.*, 2004).

A. philoxeroides reproduces both sexually and asexually. However, production of viable seeds seems to be restricted to its native range. Understanding why some populations of alligator weed are fertile and others sterile may be important in understanding why this plant is invasive and in the development of management strategies. Factors, both intrinsic, e.g. gametogenesis, and extrinsic, e.g. pollination processes, affect the ability of plants to produce seeds. Seeds and seedlings of both known forms of alligator weed were recorded in the field and in germination trials in the lab (Sosa *et al.*, 2004). Nevertheless, the requirements for successful sexual reproduction, the characterization of hybrids and their role in the invasiveness of the species remain uncertain.

All alligator weed in Argentina propagates vegetatively, and only in particular situations does it also propagate by seed. The reliance on vegetative reproduction could indicate the presence of hybrids in the native range, as in other Amaranthaceae in South America (Greizerstein and Poggio, 1992). Hybrids can be fertile or sterile, depending on the differences between the parental genomes. They can also develop an enhanced

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vegetative reproduction capacity. The strongest evidence for the presence of hybrids can be observed in cytogenetic studies, such as the characterization of meiosis (Mallet, 2005).

For biological control of alligator weed, the flea beetle, *Agasicles hygrophila* Selman and Vogt (Chrysomelidae: Alticinae), the stem borer, *Arcola malloi* (Pastrana) (Lepdidoptera: Pyralidae), and the thrips, *Amynotrips andersoni* O'Neil (Thysanoptera: Phaelothripidae), were released in the USA to control the weed. The flea beetle and the moth were subsequently introduced in Australia (Julien, 1981; Julien and Griffiths, 1999). In spite of their success, there are limitations, mainly concerning terrestrial growth of the weed and the lack of biological control in cooler areas of Australia and the USA (Cofrancesco, 1988; Julien and Bourne, 1988; Julien *et al.*, 1995; Julien and Stanley, 1999).

There are a number of reasons why the flea beetle, currently the most important of the biological control agents, may be restricted in space and habitat. Its life cycle is strongly associated with aquatic conditions, including high humidity. For successful pupation, it requires hollow stems with thin walls (normally found in aquatic growth) rather than thick-walled stems with small cavities (normally terrestrial growth; Vogt, 1973). The flea beetle is also sensitive to low temperatures (Stewart *et al.*, 1996). However, no possible relationship between its performance and plant genetic differences within alligator weed has been considered thus far.

Genetic studies were initiated to understand the morphological variation found in *A. philoxeroides* in its native range and to characterize alligator weed populations in Australia. In addition, a preliminary study was conducted for demographic parameters of the flea beetle under laboratory conditions to evaluate the role of plant ploidy level in the insect's performance.

Methods and materials

Morphological studies

Cultures from seedlings and stems from the following different localities in Argentina were cultivated in the greenhouse for 6 months under controlled conditions: 1 – Rt. 11, 22 km SW Reconquista, Santa Fé Province, 29°16′51.3″S, 59°49′12.8″W (from now on called Santa Fé); 2 – Rt. 380, to Lules, Tucumán Province, 26°52′27.5″S, 65°18′25.1″W (Tucumán); 3 – Hurlingham, Buenos Aires Province 34°35′14.2″S, 58°38′24.0″W (Hurlingham) and 4 – Rt.30, 23 km from Tandil, Buenos Aires Province, 37°11′35.5″S, 59°03′29.7″W (Tandil). Twenty-one morphological parameters were measured and analysed using a principal component analysis (PC – ord 4). The variables that explained the greater portion of the variance were analysed using a one-way analysis of variance (ANOVA), and Tukey's honestly significant difference (HSD) test was used to separate the means.

Cytogenetic studies

The cytogenetic variation of alligator weed was studied to identify hybrid forms in both the native and adventive ranges and to characterize their reproductive status (sexual or asexual). Young flowers were collected into vials with a 6:3:1 solution of 96% ethyl alcohol, chloroform and acetic acid, from 12 sites in Argentina (Fig. 1) and nine in Australia (Fig. 2). The process of meiosis in flower-bud cells was studied. In addition, mitosis was studied in at least 30 cells taken from fine root-tips. Viability of pollen from anthers was estimated using Alexander stain, a stain for chromatin. Unstained pollen grains indicate absence of chromatin and non-viability.

A. hygrophila performance in relation to ploidy levels

Adults of the flea beetle were collected from the field at Hurlingham, near Buenos Aires, and raised in a chamber at 25°C and 12-h light. Ten first-instar larvae were placed in a plastic container (8 cm diameter, 5 cm height) with moistened tissue paper and fed on plants from different alligator weed populations: Santa Fé (hexaploid), Predelta (hexaploid), Hurlingham (hexaploid), Cañuelas (hexaploid) and Tandil (tetraploid). Each treatment was replicated ten times. To estimate performance of the flea beetle on plants from the different localities, survivorship of larvae and immature developmental time were measured. The results were analysed with multivariate ANOVA (MANOVA), and Tukey's HSD test was used to compare means.

Results

Morphological studies

The first three axes of the principal component analysis explained 75% of the variance. Populations were ordered according to the variables associated to these axes: shoot diameter, width-to-length ratio of the first leaf and apical angle of the first leaf (Fig. 3). The populations that were more representative of each form (Table 1) were analysed in terms of these three variables. The Cañuelas population closely resembled Tandil population in shoot diameter, but leaves were larger and the width-to-length ratio was intermediate between Hurlingham and Santa Fé. The Predelta population, on the contrary, closely resembled Hurlingham population, although with bigger leaves and shoots.

A. philoxeroides f. *angustifolia* differs from *A. philoxeroides* f. *philoxeroides* in the diameter of the stems and in the shape of the leaves. In the laboratory, *A. philoxeroides* f. *philoxeroides* from Tandil (APP in Table 1) had significantly thinner stems (smaller

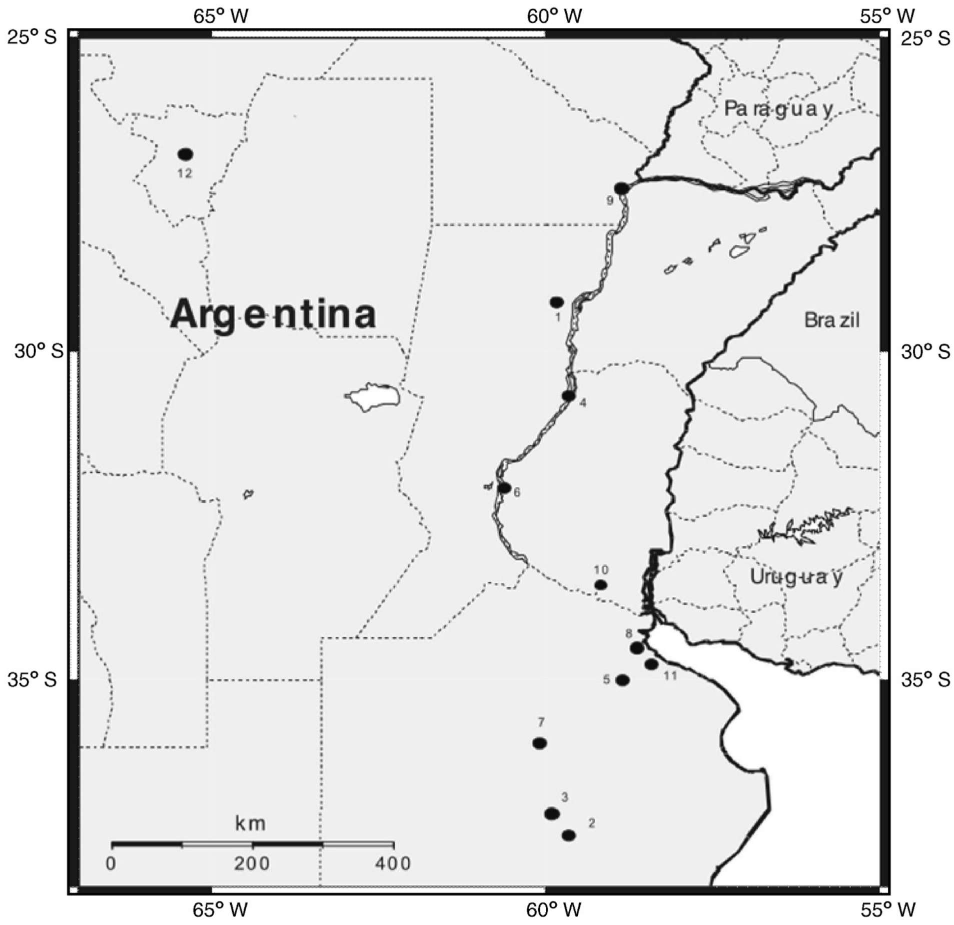


Figure 1. The locations of the 12 Argentine populations of *Alternanthera philoxeroides* that were studied. Numbers refer to populations described in Table 2.

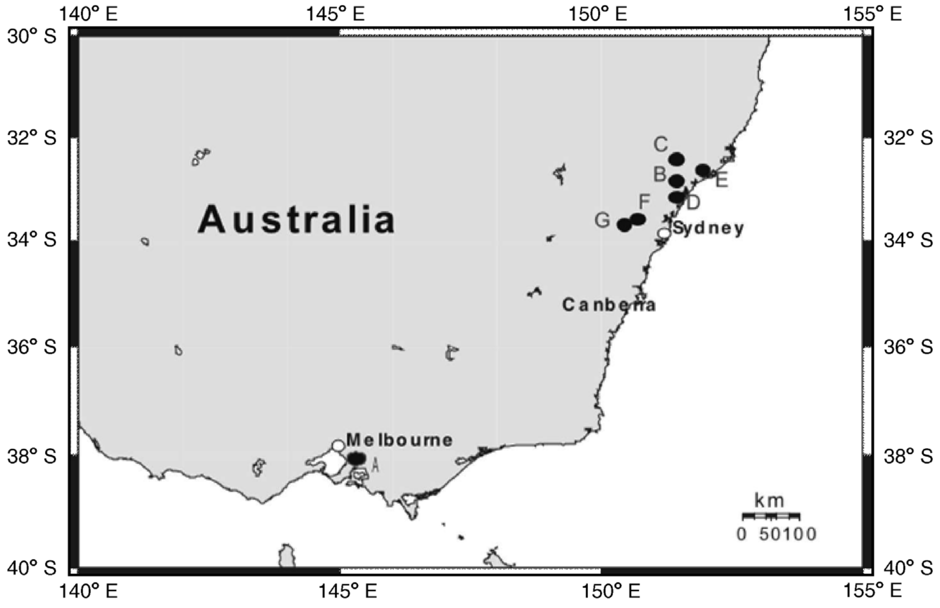


Figure 2. The locations of the nine Australian populations of *Alternanthera philoxeroides* that were studied. Letters refer to populations described in Table 2.

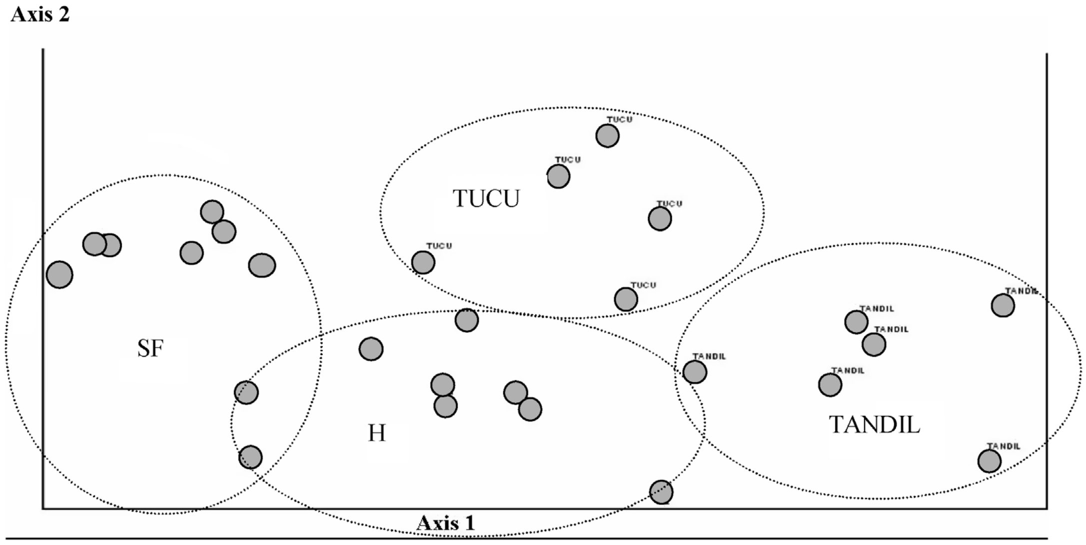


Figure 3. Ordination obtained with principal component analysis of four populations of *Alternanthera philoxeroides*; Santa Fé (*SF*), Hurlingham (*H*), Tandil (*Tandil*) and Tucumán (*TUCU*), using 21 morphological variables.

internode diameter), ovate leaf tips (greater leaf apical angle) and shorter leaves (higher values in leaf width-to-length ratio). The *angustifolia* form from Hurlingham (APA in Table 1) significantly differed in its more acute leaf apical angle, longer leaves (lower leaf width-to-length ratio) and broader stems (greater internode diameter). Plants from Santa Fé (intermediate form in Table 1) resembled the *angustifolia* form due to their broad stems, whereas they had similar leaf width-to-length ratio and leaf apical angle to the *philoxeroides* form (Table 1).

Cytogenetic studies

Alligator weed has small and numerous chromosomes that are difficult to distinguish and count. The results revealed that alligator weed populations in Argentina are composed of a complex of hybrids. Chromosome number differed among populations within the native range (Table 2). Populations from Tandil had 66 chromosomes compared to other populations with higher numbers (approximately 100). In comparison, samples from Santa Fé, Cañuelas, Tucumán and Australia (Table 2) had ‘aberrant’ meiosis in which several univalent chromosomes were not aligned in the equatorial plate. The resulting cells had different chromosome numbers, approximately 100. Material from Australian populations could not be analysed for chromosome number because root-tips were in an advanced state of cell division at the time of the study.

It is interesting to note that *Alternanthera aquatica* Chod., a species closely related to alligator weed, also had 66 chromosomes. This is the first report of a chromosome number for this plant species.

Pollen staining confirmed low viability of pollen grains, suggesting hybrid forms of *A. philoxeroides*. Pollen viability from Tandil and Predelta was higher than in Santa Fé, Cañuelas, Hurlingham or Australia (Table 2).

A. hygrophila performance in relation to ploidy levels

Preliminary results show that host genetic variability affects the performance of *A. hygrophila* (MANOVA: Wilks lambda = 0.63, P = 0.008). Differences were found in immature survivorship (Fig. 4, ANOVA: $F_{4,\ 44}$ = 4.07, P = 0.006), which was higher in plants from Hurlingham (0.77 ± 0.03) than in those from either Santa Fé (0.44 ± 0.07) or Predelta (0.49 ± 0.08),

Table 1. Morphometric data for laboratory-grown *Alternanthera philoxeroides* from three locations in Argentina. *APP*, *A. philoxeroides* f. *philoxeroides*; *APA*, *A. philoxeroides* f. *angustifolia*; *AP?*, an intermediate form. Means and standard errors are shown. Means *within a column followed by different letters* are significantly different ($P < 0.05$; ANOVA, Tukey post hoc multiple comparisons).

Form	Collecting site	Internode diameter (mm)	Leaf length to width ratio	Leaf apical angle (°)
APP	Tandil, *n* = 10	1.95 ± 1.11a	0.40 ± 0.07a	137.33 ± 43.16a
AP?	Santa Fé, *n* = 10	2.84 ± 0.77 b	0.42 ± 0.12a	105.56 ± 31.00a
APA	Hurlingham, *n* = 10	3.16 ± 0.48b	0.24 ± 0.05b	68.83 ± 14.45b

Table 2. Results of cytogenetic studies on 12 Argentine populations of *Alternanthera A. philoxeroides*. The *dash* (–) indicates that data is not yet available. The *numbers in the first column* relate to locations in Figs. 1 and 2.

Population	Number of individuals	Form	Chromosome number 2n	Pollen stainability (%)	Observations on pollen grains	Fruits
Argentina populations						
Reconquista, Santa Fé (1)	5	Intermediate[a]	Approximately 100	50	Different size (aneuploid)	No
Tandil, Buenos Aires (2)	6	*philoxeroides*	66	95	Normal morphology and size	Yes
Tandil, Buenos Aires (3)	4	*philoxeroides*	66	95	Normal morphology and size	Yes
La Paz, Entre Ríos (4)	5	*angustifolia*		61	Different size (aneuploid)	No
Cañuelas, Buenos Aires (5)	5	*angustifolia*	*ca.* 100	0		Yes
Predelta, Entre Ríos (6)	4	*angustifolia*	*ca.* 100	94	Normal morphology and size	No
Cazón, Buenos Aires (7)	4	*intermediate		65	Different size (aneuploid)	No
Hurlingham (8)	5	*angustifolia*		0		Yes?
Chaco (9)	5	*A. aquatica?*	66	15	Different size (aneuploid)	No
Paranacito (10)	5	*A. aquatica?*		0	–	No
La Plata (11)	5	*intermediate	66	–	–	Yes
Tucumán (12)	5	*angustifolia*	100	–	–	No
Australian populations						
Hunter Valley, NSW (A)	3	*angustifolia*	–	5	Different size (aneuploid)	
Dandenong, Victoria (B)	2	*angustifolia*	–	8	Different size (aneuploid)	
Kaotara, NSW (C)	4	*angustifolia*	–	–		
Maitland East, NSW (D)	3	*angustifolia*	–	8	Different size (aneuploid)	
Wallsend, NSW (E)	4	*angustifolia*	–	–		
Oakville, NSW (F)	5	*angustifolia*	–	7	Different size (aneuploidy)	
Richmond, NSW (G)	3	*angustifolia*	–	6		

[a] Intermediate: A form that appears to be intermediate between the two forms *angustifolia* and *philoxeroides* (see Table 1).

but showed no difference from plants from Tandil (0.57 ± 0.06) or Cañuelas (0.57 ± 0.06).

However, developmental time (in days) of different hybrids were not different (Santa Fé 22.2 ± 0.8; Predelta 21.1 ± 1.1; Hurlingham 21.2 ± 0.6; Tandil 23.1 ± 0.9; Cañuelas 20.4 ± 0.5; ANOVA: $F_{4,\ 44} = 1.85$, $P = 0.136$).

Discussion

Irregularities in meiosis division are associated with hybrid organisms (Mallet, 2005). Irregularities observed through cytogenetic analysis, and subsequent correlation with pollen staining strongly suggests that the entity *A. philoxeroides* is a complex of hybrids. Additional chromosomes (through hybridization) could be beneficial for these plants (Levin, 2002), particularly as they do not depend on sexual reproduction. New polyploids may possess novel physiological, ecological or phenological characteristics that allow them to colonize new niches, and they may be wholly or partially reproductively isolated from their diploid progenitors (Ramsey and Schemske, 1998).

Based on our results and knowing that in the Gomphreninae tribe the basic chromosome number is x = 16–17 (Okada *et al.*, 1985), we propose a hypothetical model showing the evolution of *A. philoxeroides* in its native area (Fig. 5). Diploid ancestors gave origin to

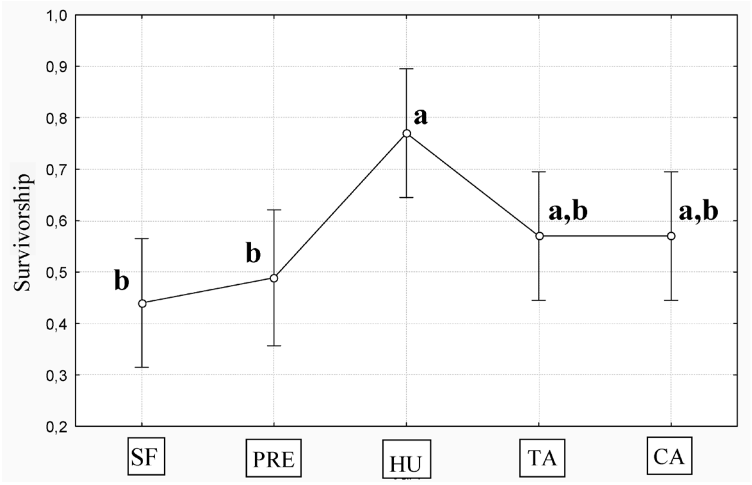
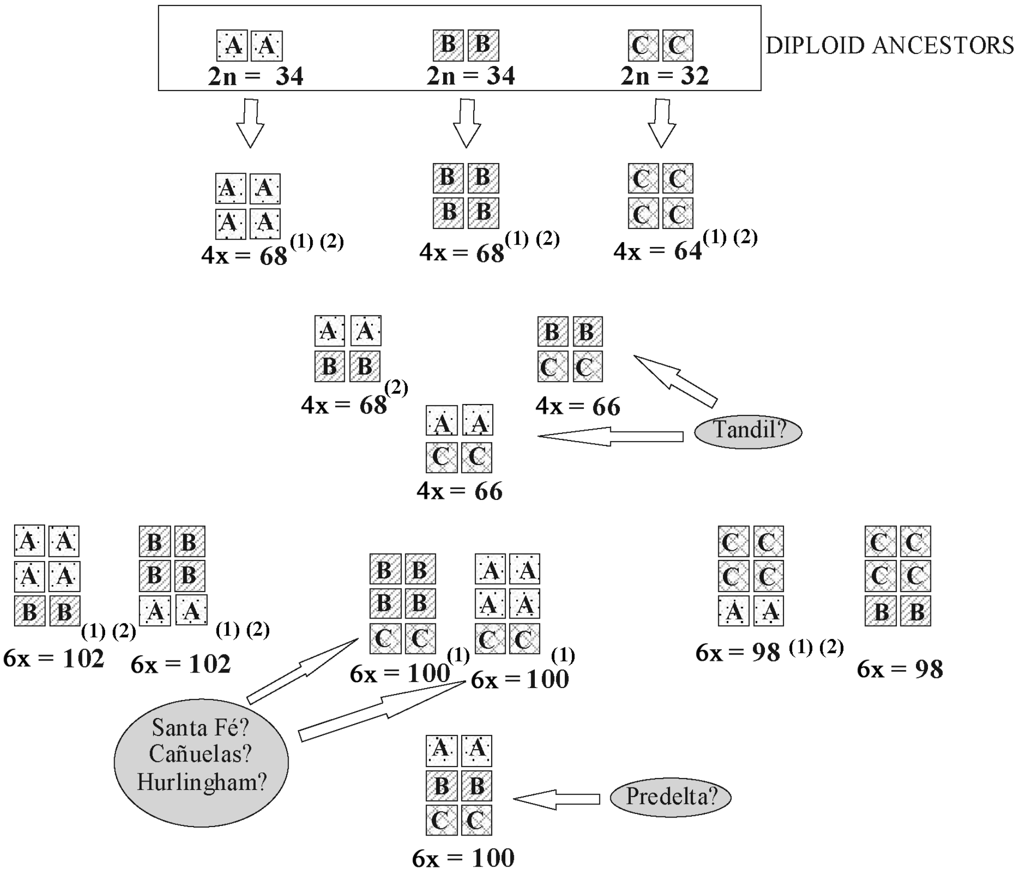


Figure 4. Survivorship of larvae of *Alternanthera hygrophila* on five populations of *A. philoxeroides* from Argentina; Santa Fé (*SF*), Predelta (*PRE*), Hurlingham (*HU*), Tandil (*TA*) and Cañuelas (*CA*). Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences (ANOVA $P < 0.05$).



(1) Aberrant meiosis due to formation of tetravalents.
(2) Not found in the field.

Figure 5. Hypothetical hybridization model of *Alternanthera philoxeroides* in Argentina.

auto- and allotetraploids, the latter being represented by the Tandil population. Due to a lack of reduction in meiosis I, these subsequently produced two different types of hexaploids, one with aberrant meiosis (Santa Fé–Cañuelas–Hurlingham) and another with normal meiosis and sexual reproduction (Predelta). However, it is still uncertain which parents gave rise to the alligator weed hybrids. To assess this issue, the genomic in situ hybridization technique will be used in the future to determine the origins of the different chromosomes in each population. This technique is used with the combination of molecular tools to study the evolutionary aspects and the role of polyploids in other biological invasions (Fehrer *et al.*, 2007). For the alligator weed, they should provide a more complete picture of its evolutionary history.

As the only cytotype of *A. philoxeroides* found in Australia is a hexaploid, we suggest that its invasive capability could be attributed to traits from one of the native range hexaploid (hybrid) populations and that these traits were gained before invasion. As previously documented for other weeds (Lee, 2002), alligator weed shows positive effects of hybridization on invasibility, such as faster growth, greater size and increased aggression (Okada *et al.*, 1985; Alonso and Okada, 1996). In addition, alligator weed plants growing in aquatic and terrestrial environments are morphologically different, and this might be due to genotype × environment interactions.

The alligator weed flea beetle that was released in the USA and Australia was collected in Buenos Aires Province in Argentina and in Montevideo, Uruguay (Coulson, 1977). The insects used in the present study were collected in Hurlingham. As it is highly probable that these populations have the same origin, the use of insects from Hurlingham for plant–herbivore interaction studies may validate comparisons between the outcome of our experiments and the behaviour of *A. hygrophila* in the adventive range. In our experiments, higher survivorship on Hurlingham plants could be explained by differences in the genotypes or by plant maternal effects. This warrants further study, and a current experiment is testing for differences in fertility and fecundity.

The presence of different cytotypes of alligator weed should be considered when explaining the lack of success of the flea beetle in controlling the weed in cool areas, particularly in the USA, where the existence of distinct alligator weed biotypes has been confirmed (Kay and Haller, 1982).

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