

A first estimation of the mating system of *Onopordum illyricum* (L.) and its relevance to the invasion of Australia

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Abstract

We tested the hypothesis that the breeding system of an invasive weed is altered following invasion using *Onopordum illyricum* (L.), a self-compatible insect-pollinated monocarpic perennial of mediterranean origin, which has invaded Australia. Our results, obtained by the analysis of the genetic composition of progeny of individual plants of an Australian population, suggest that this species has an intermediate outcrossing rate ($t=0.57$) in its area of introduction. This mixed mating system seems to be stable, since the estimates obtained by other techniques assuming equilibrium, are very close. Furthermore the mating system of this species has apparently not changed during invasion, since estimates of the outcrossing rates of French populations are similar to that of the Australian population ($t=0.52$). The mixed mating system of this species, may have contributed to its invasive capacity, as selfing allows avoidance of demographic hazards, and outcrossing would promote the generation of novel genotypes allowing adaptation to novel environmental conditions.

Keywords: Mating system, outcrossing, *Onopordum illyricum*, biological invasions.

Résumé

Nous avons testé l'hypothèse selon laquelle le régime de reproduction d'une plante envahissante est modifié suite à une invasion sur *Onopordum illyricum* (L.), une plante autocompatible entomophile et pérenne monocarpique d'origine méditerranéenne qui a envahi l'Australie. Nos résultats, obtenus par l'analyse de la composition génétique de la descendance d'individus d'une population australienne, suggèrent que cette espèce a un taux d'allofécondation intermédiaire ($t=0.57$) dans son aire d'introduction. Ce régime de reproduction mixte semble être stable, puisque des estimations obtenues par d'autres techniques qui supposent qu'un équilibre a été atteint sont très proches. De plus, le système de reproduction de cette espèce n'a apparemment pas changé pendant l'invasion, puisque les estimations du taux d'allofécondation des populations françaises ($t=0.52$) sont proches de celles de la population australienne. Le régime de reproduction mixte de cette espèce peut avoir contribué à sa capacité d'invasion, étant donné que l'autofécondation permet l'évitement de risques démographiques alors que l'allofécondation, en avantageant la formation de nouveaux génotypes, permet l'adaptation à de nouvelles conditions environnementales.

INTRODUCTION

More than twenty five years ago ALLARD (1965) and BAKER (1965) emphasized the importance of the breeding system in the ability of plants to become invasive. Both authors noted that plants with a largely autogamous plastic breeding system would be the best candidates for the colonization and invasion of new areas. According to these authors, high levels of selfing may confer two advantages during invasion, namely a decrease of demographic stochasticity (independence from the presence of other mates for reproduction), and a more efficient propagation of successful genotypes. In recent reviews BROWN & BURDON (1987) and BARRETT & RICHARDSON (1986) also emphasized the importance of the capacity of uniparental reproduction for successful colonization.

In accordance with these predictions BROWN & MARSHALL (1981) have shown that among the 18 worst weeds in the world, half are self-pollinated species while the remaining ones are outcrossers capable of vegetative reproduction. PRICE & JAIN (1981), in a compilation of approximately 400 species of the British Isles, have shown that selfing and apomixis are predominant among colonizers. In the same line of evidence, among the species compiled by SCHEMSKE & LANDE (1985), all the highly selfing species can be classified as colonizers (HERMANUTZ, 1991). Finally, in several cases it has been demonstrated that colonization of a new area by a species has been accompanied by a shift of the mating system towards increased selfing (e.g. colonization of north-eastern America by *Solanum ptycanthum* (HERMANUTZ, 1991) and other examples in BROWN & MARSHALL, 1981).

On the other hand, it has been argued that increased outcrossing may be favoured during colonization, because outcrossing allows the production of a larger number of genotypes, and therefore can potentially allow a species to cope with different environmental selective pressures (BARRETT & RICHARDSON, 1986). In agreement with this argument, shifts in the mating system following colonization towards higher rates of outcrossing, have been reported for *Bromus mollis* (BROWN & MARSHALL, 1981) and *Avena barbata* (KAHLER *et al.*, 1980). It should be noted, however, that both of these species are predominantly selfers. Other evidence in favour of this idea comes from the biological control database. BURDON & MARSHALL (1981), in their survey on biological control of weeds in Australia, have shown that biological control has been significantly more successful on asexually reproducing plants than on sexually reproducing ones.

The thistle *Onopordum illyricum* (L.) (Asteraceae: Cardueae), native to the mediterranean basin, was introduced to Australia during the nineteenth century and is now considered as a major weed (BRIESE *et al.*, 1990). As a consequence a biological control program has been engaged against it (BRIESE, 1989). *O. illyricum* is a monocarpic perennial, potentially producing up to 40,000 seeds per individual (YOUNG & EVANS, 1969). Research on this species in Australia revealed that the genetic composition of seedlings does not correspond to the one expected by random mating, showing a deficit of heterozygotes (GROVES *et al.*, 1990). Furthermore, these authors have shown that inbreeding depression must be important in this species, since the level of heterozygosity increases as a cohort ages, while the allelic frequencies remain constant (thus excluding the possibility of selection acting in favour of individual alleles).

In this paper we report the results of a study designed to estimate specifically the outcrossing rate of *O. illyricum* in Australia. We then compare the estimated outcrossing rate with estimations of the outcrossing rate by other techniques in Australia (GROVES *et al.*, 1990) and France (MICHALAKIS *et al.*, in press), to discuss the stability of the observed mating system and the relevance of the mating system for the invasion of Australia.

MATERIALS AND METHODS

Sampling

One capitulum per plant was collected from 30 plants at a site at Galong (New South Wales). This site is the same as the one in which GROVES *et al.* (1990) conducted their study. Seeds from each capitulum were germinated in Petri dishes and then were transplanted to a greenhouse. When the seedlings obtained their first true leaves, plant tissue was collected, and the genotype of each individual was scored by allozyme electrophoresis. We wanted to sample the same stage as GROVES *et al.* (1990), as it is known that enzyme activity may vary between stages of the same individual (SCANDALIOS, 1974). The seedlings of each capitulum thus constituted families. Thirty capitula were sampled, and on average five seedlings per capitulum were scored by allozyme electrophoresis.

Electrophoresis

GROVES *et al.* (1990) scored five polymorphic loci by horizontal starch gel electrophoresis. Unfortunately, even though our samples came from the same site, all those loci were monomorphic when screened by the same technique. However, two of these loci, leucine aminopeptidase (LAP; EC 3.4.11.1) and aspartate aminotransferase (AAT; EC 2.6.1.1) were polymorphic on vertical polyacrylamide gels. We have also found another polymorphic locus by the latter technique, which had not been investigated by GROVES and collaborators (Est; EC 3.1.1.3). Thus, despite our precautions on plant stage, our results cannot be directly compared with the ones obtained by GROVES *et al.* (1990). Vertical polyacrylamide gels had an 8 % concentration for LAP and AAT, and an 11 % concentration for esterase.

Data analysis

The data have been analysed by the MLT program (RITLAND, 1990; RITLAND & JAIN, 1981), which uses the genotypes of the progeny to infer the genotypes of the parental plants and the allelic composition of the pollen pool. Using this information, and the assumption of random distribution of pollen between plants, this procedure provides maximum likelihood estimates of single locus outcrossing rates, as well as a multilocus estimate, accompanied by the respective standard errors. A chi-squared test for heterogeneity among single locus estimates was performed (KAHLER *et al.*, 1984). The value of this χ^2 is equal to

$$\chi^2 = \sum_i^n (t_i - \bar{t})^2 I_i$$

where \bar{t} is the mean outcrossing rate and t_i and I_i are the single locus estimate and the inverse of its variance respectively, for locus i . The number of degrees of freedom is equal to $n-1$, where n is the number of loci.

RESULTS

Single locus estimates of the outcrossing rate range from 0.5 to 0.6 (table I). No significant heterogeneity was found among the single locus estimates ($\chi^2_2 = 0.53$).

TABLE I. - Number of seedlings scored per locus (n), single locus (t_i), multilocus (t_m) and mean single locus (t) outcrossing rate estimates. Standard errors are given in parentheses.

Locus	n	t_i
AAT	147	0.597 (0.103)
LAP	149	0.502 (0.135)
EST	137	0.501 (0.108)
t		0.533
t_m		0.568 (0.068)

TABLE II. - Outcrossing rates (t) of 8 French populations calculated from F_{is} values (original data in MICHALAKIS *et al.*, in press) n_H : harmonic mean of per locus sample size; CI: 95 % confidence intervals obtained by bootstrapping over loci (WEIR, 1990).

Population	n_H	t	CI
V1	38.49	0.62	0.53-0.71
V2	30.87	0.64	0.24-1.10
V3	35.75	0.55	0.44-0.60
V4	44.80	0.34	0.25-0.45
V5	34.97	0.61	0.43-0.78
V6	53.50	0.59	0.45-0.69
V8	36.43	0.55	0.44-0.67
CR	32.30	0.81	0.53-1.15

The multilocus estimate is intermediate and equal to 0.57. The difference between the mean single locus estimate (t) and the multilocus estimate (t_m) is very small ($\Delta t = t_m - t = 0.035$).

HALDANE (1924) has shown that, at equilibrium, the deficit of heterozygotes within a population (measured by WRIGHT's fixation index F_{is} , WRIGHT, 1951) is related to the outcrossing rate (t) by the relationship:

$$t = \frac{1 - F_{is}}{1 + F_{is}}$$

In their study of this species at the same site in Australia, GROVES *et al.* (1990) estimated F_{is} at 0.30. Thus we can estimate the outcrossing rate in their samples, using the above equation, to be equal to 0.54. This value is very close to the multilocus estimate for the same population, which makes no assumptions about equilibrium.

We have also calculated F_{is} in populations in southern France using similar techniques (MICHALAKIS *et al.*, in press). In this case, the average F_{is} across loci and populations is equal to 0.32 and yields an estimated outcrossing rate of 0.52. Thus, the outcrossing rate of French populations is very close to the one for the Australian population. However, the French populations show considerable variation in the estimates of the outcrossing rate (table II).

DISCUSSION

Three independent measures of the outcrossing rate of *O. illyricum*, using different estimation techniques and different genetic markers, show that this

plant species has an intermediate outcrossing rate (approximately 0.55). Mixed mating systems may represent either transient states (LANDE & SCHEMSKE, 1985) or evolutionarily stable states (HOLSINGER, 1988 and 1991; UYENOYAMA, 1986; UYENOYAMA & WALLER, 1991 *a, b*). The fact that the estimate obtained by the MLT method, which makes no assumptions about equilibrium, is almost identical to the estimate obtained using the F_{is} value (from GROVES *et al.*, 1990), which assumes that the population at Galong is at equilibrium, argues for the stability of the mating system of *O. illyricum*. The very close value of the estimate obtained in the French populations reinforces this conclusion.

Stable mixed mating systems can be theoretically generated by biparental inbreeding (UYENOYAMA, 1986), inbreeding depression caused by overdominance (HOLSINGER, 1988; UYENOYAMA & WALLER, 1991 *a, b*) or environmental effects (such as plant density) on the reproductive success of outcrossed pollen in self-compatible plants (BROWN *et al.*, 1989; WATKINS & LEVIN, 1990; HOLSINGER, 1991). The thistle *O. illyricum* is a self-compatible, insect-pollinated plant. GROVES *et al.* (1990) have shown that inbreeding depression is important in this species, since heterozygosity increases as a cohort ages while individual allele frequencies stay constant. This inbreeding depression does not seem to be caused by biparental inbreeding. Indeed, SHAW *et al.* (1981) have shown that biparental inbreeding would create a difference between the single locus and the multilocus estimates of the outcrossing rate. In the case of the Galong population this difference is very small (table I).

We clearly need more studies on populations in Australia, since outcrossing rates may vary geographically or according to plant density. Based on the comparison of the outcrossing rate of the Galong population and the populations from southern France we could conclude that the invasion of Australia has not been accompanied by a shift in the breeding system. Indeed, the outcrossing rates are very similar. However, in as much as our results are representative of the species, the nature of the mating system of *O. illyricum* suggests that the reproductive mode may have had considerable influence on the colonization process. BAKER (1965) and ALLARD (1965) stress the importance of the flexibility of the breeding system. They consider that the best candidates for colonization are species which have both appreciable levels of selfing, and variable levels of selfing across populations. This weed presents both of these characters, since the outcrossing rate of French populations varies from 0.34 to 0.81. The amount of selfing combined with the capacity of this plant to produce large quantities of seeds probably protects this species from demographic stochasticity. The level of outcrossing on the other hand, is probably sufficient to generate novel genotypes and allow a rapid adaptation to new environmental conditions, thus increasing the invasive ability of this species.

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REFERENCES

- ALLARD R. W., 1965. – Genetic systems associated with colonizing ability in predominantly self-pollinated species. In: BAKER H. G. & STEBBINS G. L., eds., *The genetics of colonizing species*. Academic Press, NY, 46-76.
- BAKER H.G., 1965. – Characteristics and modes of origin of weeds. In: BAKER H.G. & STEBBINS G.L., eds., *The genetics of colonizing species*. Academic Press, NY, 147-172.
- BARRETT S. C. H. & RICHARDSON B. J., 1986. – Genetic attributes of invading species. In: GROVES R. H. & BURDON J. J., eds., *Ecology of biological invasions*, Cambridge University Press, Cambridge, 21-33.
- BRIESE D. T., 1989. – A new biological control program against thistles of the genus *Onopordum* in Australia. In: DELFOSSE E. S., ed., *Proceedings of the VII International Symposium of Biological Control of Weeds*, MAF, Rome/CSIRO, Melbourne, 155-163.
- BRIESE D. T., LANE D., HYDE-WYATT B. H., COKER J. & DIVER R. G., 1990. – Distribution of thistles of genus *Onopordum* in Australia. *Plant Protection Quarterly*, 5, 23-27.
- BROWN A. H. D. & BURDON J. J., 1987. – Mating systems and colonizing success in plants. In: GRAY A. J., CRAWLEY M. J. & EDWARDS P. J., eds., *Colonization succession and stability*, Blackwell, Oxford, 115-131.
- BROWN A. H. D. & MARSHALL D. R., 1981. – Evolutionary changes accompanying colonization in plants. In: SCUDDER G. G. E. & REVEAL J. L., eds., *Evolution today, Proc. II Int. Congress Syst. and Evol. Biol.*, Hunt Institute for Botanical documentation, Carnegie-Mellon University, Pittsburgh, 351-363.
- BROWN A. H. D., BURDON J. J. & JAROSZ A. M., 1989. – Isozyme analysis of plant mating systems. In: SOLTIS D. & SOLTIS P., eds., *Isozymes in plant biology*, Dioscorides Press, Portland, OR, 73-86.
- BURDON J. J. & MARSHALL D. R., 1981. – Biological control and the reproductive mode of weeds. *J. Appl. Ecol.*, 18, 649-658.
- GROVES R. H., BURDON J. J. & KAYE P. E., 1990. – Demography and genetics of *Onopordum* in southern New South Wales. *J. Ecol.*, 78, 47-55.
- HALDANE J. B. S., 1924. – A mathematical theory of natural and artificial selection. II. The influence of partial self-fertilisation, inbreeding, assortative mating and selective fertilisation on the composition of mendelian populations. *Camb. Phil. Soc. Trans.*, 1, 158-163.
- HERMANUTZ L., 1991. – Outcrossing in the weed, *Solanum ptycanthum* (Solanaceae): a comparison of agrestal and ruderal populations. *Amer. J. Bot.*, 78, 638-646.
- HOLSINGER K. E., 1988. – Inbreeding depression doesn't matter: the genetic basis of mating system evolution. *Evolution*, 42, 1235-1244.
- HOLSINGER K. E., 1991. – Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *Am. Nat.*, 138, 606-622.
- KAHLER A. L., ALLARD R. W., KRZAKOWA M., WEHRHAIN C. F. & NEVO E., 1980. – Associations between isozyme phenotypes and environment in the slender wild oat (*Avena barbata*) in Israel. *Theoret. Appl. Genet.*, 56, 31-47.
- KAHLER A. L., GARDNER C. O. & ALLARD R. W., 1984. – Nonrandom mating in experimental populations of maize. *Crop Sci.*, 24, 350-354.
- LANDE R. & SCHEMSKE D. W., 1985. – The evolution of self-fertilisation and inbreeding depression in plants. I. Genetic models. *Evolution*, 39, 24-40.
- MICHALAKIS Y., SHEPPARD A. W., NOEL V. & OLIVIERI I., in press. – Population structure of a herbivorous insect and its host plant on a microgeographic scale. *Evolution*.
- PRICE S. C. & SAIN S. K., 1981. – Are inbreeders better colonizers? *Ecologia*, 49, 283-286.
- RITLAND K., 1990. – A series of FORTRAN computer programs for estimating plant mating systems. *J. Heredity*, 81, 235-237.
- RITLAND K. & JAIN S. D., 1981. – A model for the estimation of outcrossing rate and gene frequencies using n independent loci. *Heredity*, 47, 35-52.
- SCANDALIOS J. G., 1974. – Isozymes in development and differentiation. *Ann. Rev. Plant. Physiol.*, 25, 225-258.
- SCHEMSKE D. W. & LANDE R., 1985. – The evolution of self-fertilisation and inbreeding depression in plants. II. Empirical observations. *Evolution*, 39, 41-52.
- SHAW D. V., KAHLER A. L. & ALLARD R. W., 1981. – A multilocus estimator of mating system parameters in plant populations. *Proc. Natl. Acad. Sci. USA*, 78, 1298-1302.

- UYENOYAMA M. K., 1986. – Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. *Evolution*, **40**, 388-404.
- UYENOYAMA M. K. & WALLER D. M., 1991a. – Coevolution of self-fertilization and inbreeding depression. II. Symmetric overdominance in viability. *Theoret. Pop. Biol.*, **40**, 47-77.
- UYENOYAMA M. K. & WALLER D. M., 1991b. – Coevolution of self-fertilization and inbreeding depression. III. Homozygous lethal mutations at multiple loci. *Theoret. Pop. Biol.*, **40**, 173-210.
- WATKINS L. & LEVIN D. A., 1990. – Outcrossing rates as related to plant density in *Phlox drummondii*. *Heredity*, **65**, 81-89.
- WEIR B. S., 1990. – *Genetic data analysis*. Sinauer, Sunderland MA, 377 p.
- WRIGHT S., 1951. – The genetical structure of populations. *Ann. Eugen.*, **15**, 323-354.
- YOUNG J. A. & EVANS R. A., 1969. – Control and ecological studies of Scotch thistle. *Weed Science*, **17**, 60-63.