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# Mating system of *Hedysarum spinosissimum* L., ssp. *capitatum* Desf, Asch et Gr. (Leguminosae, Fabaceae)

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**RESUME** – "Système de croisement de *Hedysarum spinosissimum* L., ssp. *capitatum* Desf. Asch et Gr. (Leguminosae, Fabaceae)". La présente étude apporte des estimations sur les paramètres des systèmes de croisement chez deux populations naturelles de *Hedysarum capitatum*, en utilisant les méthodes des allozymes par électrophorèse. Les taux de croisement éloigné ont été estimés en utilisant les semences testées sur deux loci polymorphes. Les taux de croisement éloigné multiloci ( $t_m$ ) variaient de  $0,796 \pm 0,096$  à  $0,808 \pm 0,073$  et étaient significativement différents du croisement éloigné complet. Les estimations de croisement éloigné pour un seul locus ( $t_s$ ), variaient de  $0,728 \pm 0,091$  à  $0,801 \pm 0,070$ , donc différaient également significativement de l'unité et étaient légèrement inférieurs à leur  $t_m$  correspondant. Cependant, le taux d'endogamie biparentale ( $s_b$ ), indiqué par la différence entre  $t_m$  et  $t_s$ , n'était significativement différent de zéro que chez une population ( $s_b = 0,068 \pm 0,026$ ).

**Mots-clés** : *Hedysarum capitatum*, système de croisement, taux de croisement éloigné, endogamie biparentale.

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## Introduction

Appropriate knowledge of the mating system permits assessment of potential problems with the regeneration of natural and *ex situ* populations. For example, small populations of self-incompatible plant species may not obtain sufficient pollen for maximum seed set if there is a small number of reproductive individuals or cross-compatible plants, or if there is variation in phenology among individuals (Whisler and Snow, 1992). In addition, self-compatible plants that typically outcross may experience higher rates of selfing and consequently higher inbreeding depression in small populations (Eckert and Barrett, 1994). Population size enhancement, hand-pollination and pollinator introductions could be utilized to ameliorate reproductive difficulties.

Plant mating systems are often described by the "mixed mating model", where a fraction of progeny are derived from self-fertilization and the remainder derived from outcrossing at random (Fyfe and Bailey, 1951). Many improvements and elaborations of the original mixed mating model have been made: Brown and Allard (1970) have shown how maternal parentage could be inferred from progeny arrays; Shaw *et al.* (1980) and Ritland and Jain (1981) introduced multilocus procedures for estimation of outcrossing. Compared with single-locus methods, multilocus methods have lower statistical variance and higher robustness against the violation of model assumptions (Ritland and Jain, 1981; Ritland, 2002). Moreover, comparisons of single with multilocus outcrossing estimates indicate levels of mating between relatives (or biparental inbreeding) (Shaw *et al.*, 1980). The later form of mating causes apparent selfing or increased homozygosity, relative to random mating. Localized seed dispersal, coupled with local pollen transfer, can lead to the development of population subdivision and biparental inbreeding (Godt and Hamrick, 1995).

*Hedysarum spinosissimum* ssp. *capitatum* (Leguminosae, Fabaceae) is an allogamous self-compatible herb, widely distributed in the western Mediterranean basin (Baatout, 1982; Baatout *et al.*, 1990). There is an increasing interest in the conservation and utilisation of *H. capitatum* genetic resources; but knowledge is limited concerning the mating system of this subspecies. Previous studies of floral biology and hand pollination (Baatout, 1982) do not provide quantitative measures of the extent of inbreeding occurring, either through selfing or biparental inbreeding, within natural populations.

The aim of the present study was to characterize the mating system in two natural populations of

*H. capitatum*, in terms of population outcrossing rates and biparental inbreeding. Progeny array data based on allozymes were used under the mixed-mating model of Fyfe and Bailey (1951).

## Materials and methods

Samples of open-pollinated seeds were collected in summer 2000 from two natural populations of *H. capitatum* located in the Bizerte (population Ghornata) and Ariana (population Nahli) regions. Fourteen to sixteen maternal plants were sampled in each population. Plants were selected on the basis of the presence of an adequate seed production, with the additional criterion that they were separated from each other by at least 1 m. About ten fruits (pods) were randomly harvested from every maternal plant. This ensured that pods were sampled from several different positions along fruiting stems. All seeds from a maternal family were pooled, and a random sample of twenty seeds was scarified and soaked in water for 24 h before being germinated for five-seven days on moist filter paper. At least nine seedlings per family were used for isozyme study. Entire seedlings were separately ground in 100  $\mu$ l of an extraction buffer described in Yagoubi and Chriki (2000). After centrifugation, enzyme extracts were individually absorbed onto chromatography paper wicks that were stored at  $-20^{\circ}\text{C}$  until needed for electrophoresis.

Electrophoresis was performed using 12% starch (Sigma) gels. Gels were stained for two enzyme systems (PGI and SKD) to resolve two polymorphic allozyme *loci*: shikimate dehydrogenase (Skd, EC 1.1.1.25) and phosphogluco-isomerase (Pgi-2, EC 5.3.1.9). Morpholine-citrate buffer system at pH 6,1 (Wendel and Weeden, 1989) was used for resolving both *loci*. Mendelian inheritance at these *loci* was verified by the conformity of progeny array patterns to Mendelian predictions, a useful method when controlled crosses are not available (Sun and Ritland, 1998).

Mean single-locus ( $t_s$ ) and multilocus ( $t_m$ ) outcrossing rates were estimated for each population, using the MLTR computer program of Kermit Ritland, currently available at: <http://www.genetics.forestry.ubc.ca/ritland/programs>. The difference between the two values ( $t_m - t_s$ ) indicates the magnitude of the biparental inbreeding ( $s_b$ ). Standard errors for the outcrossing and biparental inbreeding rates were generated using 1000 bootstrap estimates with resampling among families.

## Results and discussion

The  $t_m$  and  $t_s$  values varied from  $0.796 \pm 0.096$  (population Ghornata) to  $0.808 \pm 0.073$  (population Nahli) and from  $0.728 \pm 0.091$  (population Ghornata) to  $0.801 \pm 0.071$  (population Nahli), respectively (Table 1). These outcrossing rates differ significantly from one in both populations of *H. capitatum*. The lowest values of outcrossing rates were obtained in population Ghornata. However, on the basis of 95 per cent confidence intervals, outcrossing rate estimates did not differ significantly among populations. In self-compatible species, post-pollination mechanisms (favoring outcross pollen and/or against selfed zygotes) may be buffering the mating system against ecological factors that influence pollen transfer (Eckert and Barrett, 1994). This may explain the narrow range of outcrossing rates ( $t_m$ : 0.796–0.808;  $t_s$ : 0.728–0.801) between populations in *H. capitatum*.

Table 1. Mean single-locus ( $t_s$ ) and multilocus ( $t_m$ ), outcrossing rates, and biparental inbreeding rate ( $s_b$ ) for two natural populations of *H. spinosissimum*, ssp. *capitatum*

Population	$t_m$ (SE)	$t_s$ (SE)	$s_b = (t_m - t_s)$ (SE)	DOC
Ghornata	0.796 (0.096)	0.728 (0.091)	0.068 (0.026)	159 (16)
Nahli	0.808 (0.073)	0.801 (0.070)	0.007 (0.014)	153 (14)

DOC indicates the total number of seeds, with the number of families in parentheses; Standard errors (SE) appear in parentheses.

The biparental inbreeding rate ( $s_b = t_m - t_s$ ) was close to zero in the population Nahli ( $s_b = 0.007 \pm 0.014$ ) and significantly greater than zero in the population Ghornata ( $s_b = 0.068 \pm 0.026$ ; Table 1). The combined effects of limited seed dispersal and near-neighbor pollination may cause biparental

inbreeding. In *H. capitatum*, flowers are pollinated by native bee species (especially *Apis mellifera*) (A.C., personal observation). Bees travel predominantly to near-neighbors. These neighbors may be related, if seed dispersal is restricted and populations differentiated. The significance of  $s_b$  in the population Ghornata may be due to the relatively small size of this narrow roadside population. The difference " $t_m-t_s$ ", as suggested by Lu (2000), is useful to compare biparental inbreeding rates of populations of the same species using the same set of *loci*.

In conclusion, estimation of the outcrossing rate revealed that *H. spinosissimum*, ssp. *capitatum* predominantly exhibits random mating on the two natural sites studied, and maintains low levels of facultative selfing. The inbreeding found in the present study appears to be size-dependent. Significant biparental inbreeding was detected in the small-size population Ghornata; but only uniparental inbreeding (self-fertilization) was found in the large population Nahli.

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