Influence of genotype-temperature interaction on pollen performance

Hedhly, A.^{1,3}, Hormaza, J.I.² & Herrero, M.^{1,3}

¹ *Estación Experimental de Aula Dei, CSIC. Apartado 202, 50080, Zaragoza, Spain.*

² *Estación Experimental La Mayora, CSIC. 29750, Algarrobo-Costa, Málaga, Spain.*

³ *Unidad de Fruticultura, CITA-DGA. Apartado 727, 50080, Zaragoza, Spain.*

Correspondence: Afif Hedhly. Departamento de Pomología, EEAD, SCIC. Apartado 202, 50080, Zaragoza, Spain.

Tel.: +34 976 716125; Fax: +34 976 716145;

e-mail: ahedhly@eead.csic.es

ABSTRACT

Pollen competition and selection has significant evolutionary consequences, but very little is known on how they can be modulated. We have examined in cherry (*Prunus avium* L.) how pollen performance is affected by the genotype of the pollen and by the environmental conditions where it grows, namely the pistilar tissue and temperature. The different pollen donor genotypes tested in this work differed in their behaviour both *in vitro* and *in vivo* and this behavior was modulated depending on the female recipient where they grew. Furthermore, there was a significant temperature-genotype interaction that affected the pollen tube population census that succeeded to reach the base of the style. The combination of these three factors, while enabling a capacity of response to variations in environmental pressures, could maintain variability in pollen performance avoiding the fixation of the genes that control pollen tube growth rate.

Keywords: pollen performance; pollen tube growth; *Prunus avium*; Rosaceae; success ratio; temperature stress.

Running title: Variation in pollen performance

INTRODUCTION

Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed producing plants. Failure in pollen performance diminishes the fitness of the male parent through a reduction on its contribution to the next sporophytic generation. Differences in pollen performance could also favor reproductive isolation, by the promotion of conspecific pollen (Carney *et al.*, 1996; Campbell *et al.*, 2003) and, conversely, could be a way to avoid inbreeding depression promoting foreign pollen (Bateman, 1956; Hauser & Siegismund, 2000). Both of them modulated through pollen tube growth rate and pollen tube attrition.

But modulation of pollen performance could also play a major part regulating gene flow and could have significant evolutionary consequences in terms of sexual and gametophytic selection. Sexual selection has been widely documented in animals (Birkhead & Moller, 1998) and there are evidences that it also takes place in plants (Arnold, 1994; Morgan, 1994; Willson, 1994; Skogsmyr & Lankinen, 2002, Stephenson & Bertin, 1983) although the subject is controversial (Grant, 1995). According to Arnold (1994), the unifying concept for sexual selection in plants and animals would be mating success. Following this interpretation, sexual selection in plants could take place at the prepollination and postpollination levels (Stephenson & Bertin, 1983). At the prepollination level, the factors involved in sexual selection include, among others, the amount of pollen produced and the differences among genotypes in flower quantity and attractiveness for pollinating agents. At the postpollination level, both male competition and female choice can be involved in sexual selection (Snow & Spira, 1991; Marshall, 1998; Mitchell & Marshall, 1998). The variation in microgametophyte populations is critical for evaluating the prevalence and potential evolutionary significance of gametophytic competition (Herrera, 2002), which is a prerequisite for gametophytic selection to occur (Hormaza & Herrero, 1994). The presence of a large overlap in gene expression

between the sporophyte and the gametophyte, the two alternating generations of plants (Mascarenhas, 1989), provides a nexus between selection occurring in both generations (Hormaza & Herrero, 1992). It has been proposed that gametophytic selection could represent a main drive in angiosperms evolution and adaptation (Mulcahy, 1979).

The prezygotic stage is emerging as a scenario for the regulation of mating (Herrero, 2003) with clear evolutive consequences both in plants and animals (Bernasconi *et al.*, 2004). Paternity analyses are becoming a powerful tool to investigate competition and selection during the reproductive phase (Bernasconi, 2003). However the results are often difficult to interpret due to the strong influence that the maternal environment may have on embryo development and survival (Havens & Delph, 1996) and the difficulties to separate paternal and maternal influences. In this sense, the study of pollen performance during the progamic phase may help to disentangle prezygotic and postzygotic effects.

Pollen performance is clearly affected by the genotype of the pollen (Snow & Spira, 1991). It has been suggested that pollen competition should eventually lead to selection for rapid pollen tube growth and, hence, to the fixation of the traits controlling pollen performance (Walsh & Charlesworth, 1992). However, several lines of evidence suggest that the genotype of the female recipient can limit the likelihood of that fixation (Mulcahy *et al.*, 1996; Hormaza & Herrero, 1996, 1999) and could play a clear role controlling pollen tube growth (Willson & Burley, 1983; Stephenson & Bertin, 1983; Herrero & Hormaza, 1996).

On the other hand, studies in a few species have shown that environmental conditions affect pollen development (Young & Stanton, 1990; Jóhannsson & Stephenson, 1998) as well as pollen tube growth (Stephenson *et al.*, 1992). If this effect is widespread in plants, it may have important evolutionary consequences. One of the most important environmental factors that could affect pollen performance is the temperature during the progamic phase. It has been shown that temperature affects pollen germination (Elgersma *et al.*, 1989; Shivanna *et al.*,

1991), and pollen tube kinetics in the style (Lewis, 1942; Jefferies *et al.*, 1982; Elgersma *et al.*, 1989). By pollinating a female genotype with pollen from two different donor genotypes, it has been recently shown that, besides those physiological effects, temperature also affects pollen tube population dynamics and that the response to temperature during the reproductive phase is genotype-dependent (Hedhly *et al.*, 2004). In this work, we study to which extent this may be a general phenomenon by testing in a larger number of genotypes the contribution to pollen performance of the genotype of the pollen donor, the genotype of the female recipient and temperature.

Work has been performed in cherry (*Prunus avium* L.) a species especially well suited for pollen competition studies for its high pollen-ovule ratio (Hormaza & Herrero, 1996). To evaluate the effect of temperature on the pollen genotype we tested two temperature regimes, which reflect cool (15°C) and hot (30°C) conditions during cherry blooming. We carried out first an *in vitro* evaluation of pollen performance of the different genotypes. Second, we carried out an *in vivo* evaluation of pollen from those genotypes in one female recipient. Finally, to test the possible effect of the female recipient, we evaluated the performance of a single pollen donor in different genotypes under the two temperature regimes.

MATERIALS AND METHODS

Plant material

This work was carried out on a collection of sweet cherry (*Prunus avium* L.) cultivars, maintained at the SIA-DGA experimental orchards located at the ‘Campus de Aula Dei’ in Zaragoza, Spain. The cultivars were chosen to maximize genetic variability taking into account their compatibility relationships, geographic origin and molecular similarity (Wünsch & Hormaza, 2002). The genotypes studied were ‘Bing’ and ‘Stark Hardy Giant’ from USA,

‘Van’ from Canada, ‘Hedelfingen’ from Germany, ‘Blanca de Provenza’, ‘Burlat’ and ‘Reverchon’ from France and ‘Talaguera Brillante’ and ‘Ambrunés’ from Spain.

***In vitro* pollen germination**

Pollen from the nine genotypes studied was obtained one day before anthesis from flowers collected randomly from three clonally propagated trees per cultivar. Anthers were removed and left to dry on a piece of paper for 24-48 hours at room temperature. Pollen was sieved through a 0.26 µm mesh. Immediately after, *in vitro* germination was carried out at 15°C and 30°C in polystyrene Petri dishes (35 by 10 mm) scattering pollen on a solidified germination medium consisting of 0.3 M sucrose, 0.6 mM calcium nitrate, 1.6 mM boric acid, and 0.8% (w/v) agar (Hormaza *et al.*, 1996). Pollen germination was arrested after 24 hours at 15°C and 3 hours at 30°C, by an immediate freezing at –20°C. This procedure has revealed to be a highly efficient method to arrest pollen germination while preserving the material for further evaluation. Prior experiments evaluating pollen germination at different times showed that incubation for 24 hours was sufficient to obtain the maximum pollen germination at each incubation temperature. One day before observation under the microscope, the frozen Petri dishes were thawed at 4°C. Pollen was scored as germinated when the length of the pollen tube exceeded the diameter of its pollen grain. For each treatment, germination was recorded in five Petri dishes by counting complete fields until reaching at least 100 pollen grains in each plate.

***In vivo* pollen germination and pollen tube growth**

Pollination procedure

Two experiments were performed, one to evaluate the performance of pollen from 8 cultivars on one female recipient, ‘Bing’, and the other to evaluate the performance of a single pollen

donor, 'Bing', on 8 different female recipients. The cultivar 'Bing' was used as a male donor due to its high pollen performance *in vitro*. To maintain uniformity we used also the same genotype as the female recipient. Flowers were collected randomly from 3 trees per cultivar, emasculated at balloon stage, one day before anthesis, and placed in trays with soaked florist foam at room temperature in the laboratory (the average temperature was 20°C). The following day, 10 flowers per treatment were pollinated and, immediately, placed in the controlled temperature chambers at 15°C and 30°C. To compare pollen performance *in vivo* and *in vitro*, pollination *in vivo* was performed at the same time and with the same batch of pollen, collected at the same time, as the pollen used in the *in vitro* test. Pollination was carried out by brushing gently the stigmas with a paintbrush loaded with pollen.

Determination of the stigmatic area

To evaluate the possible effect that the stigmatic area may have on pollen adhesion and germination, the stigmatic areas of the different female genotypes were measured in the experiment of 8 female recipients pollinated by one male donor. Flowers were fixed in formalin: acetic acid: 70% ethanol (1: 1: 18; FAA) (Johansen, 1940) 48 hours after pollination. The day before stigmatic area determination, the pistils were rehydrated by washing with distilled water 3 times, one hour each, and maintained in distilled water overnight. The following day, they were hold flat on wet florist foam and observed under a Wild Heerbrugg M8 binocular microscope (Wild Heerbrugg, Switzerland). Images were transferred to a Quantiment 570 image analysis system (Leica, Cambridge, UK) through a Cohu 8310 RGB colour camera (Cohu, San Diego, Calif., USA).

Microscopic observation

Pollen grain adhesion, germination, and pollen tube growth were monitored in squash preparations. For this purpose, pistils were transferred to a 5% Na₂SO₄ solution for 4 hours, autoclaved at 1.1 kg/cm² during 15 min (Jefferies & Belcher, 1974), and stained with 0.1% aniline blue in 0.03 M K₃PO₄ (Linskens & Esser, 1957). Preparations were examined under an Ortholux II microscope (Leitz, Wetzlar, Germany) equipped with UV epifluorescence with a Band Pass 355-425 exciter filter and an LP 460 barrier filter.

In each flower, the numbers of adhered and germinated pollen grains were counted and the percentage of pollen germination calculated. Likewise, the number of pollen tubes reaching the base of the style was also counted. To express pollen tube performance in the style, the success ratio was defined as [(number of pollen tubes at the base of style / number of germinated pollen grains) X 1000] (Hedhly *et al.*, 2004).

Statistical analysis

For statistical analysis, percentage data (germination percentage and success ratio) were subjected to arcsine root square transformation. Prior to ANOVA, the data were tested for normality using the Kolmogorov-Smirnoff's test and for homogeneity of variances with the Bartlett test. The experimental design for all dependent variables was double factorial (donor/recipient, temperature) with ten repetitions. An analysis of variance by the general linear model (GLM) procedure was performed using SAS GLM v. 8 (SAS Institute Inc., Cary, N.C.), and, due to some missing values, type III sum of squares was interpreted. An analysis of covariance was carried out for the experiment of 8 female recipients pollinated by one pollen donor, taking the stigmatic area as a covariable. Different models of factor combinations with the covariable were examined (heterogeneity of slopes) (Silknitter *et al.*, 1999) although only the model in which the covariable and its interactions could explain some

variation is presented; otherwise just the ANOVA is shown. In case of significant genotype-temperature interactions and for the analysis of covariance, means separation was carried out using the lsmeans option. In case of no significant interaction in the analysis of variance, Duncan's multiple range test was used for means separation.

RESULTS

In vitro pollen germination

The effect of two constant temperature regimes (15°C and 30°C) on the *in vitro* pollen germination of 9 sweet cherry genotypes was evaluated and expressed as the percentage of germinated pollen grains (Fig. 1). The analysis of variance (Table 1) revealed a highly significant effect of the main factors (temperature and pollen genotype) and a significant effect of the pollen genotype-temperature interaction. While differences between genotypes at both temperatures are clear, high temperature reduced pollen germination and this reduction was significant for several genotypes.

Performance of different pollen donors in one female recipient

To evaluate to which extent these genotypic differences and the effect of temperature recorded *in vitro* were consistent *in vivo*, the same batch of pollen from the 8 donors was used to pollinate at the same time *in vivo* a single cultivar, 'Bing'. Self pollen was omitted due to the self-incompatibility of this cultivar.

The GLM procedure (Table 2) revealed a significant effect of the two main factors, the donor genotype and temperature, for all the independent variables (number of adhered pollen grains, number of germinated pollen grains, percentage of germination and success ratio). However, the donor genotype-temperature interaction was significant only for the number of adhered pollen grains and the success ratio.

Both the number of adhered and germinated pollen grains differed significantly among the pollen donor genotypes (Table 2) and these two parameters were significantly correlated ($r = 0.74$, $n = 157$, $P < 0.001$). This may be related to the fact that small differences were recorded among the pollen donors in the percentage of pollen germination (Fig. 2a). In spite to the fact that the same pollen was placed at the same time both in the stigmas and in the germination medium, pollen performance *in vivo* and *in vitro* did not correlate. Overall, *in vitro* pollen germination was slightly higher than that obtained *in vivo*. Moreover, the ranking order of the different pollen donors between *in vitro* and *in vivo* germination was not maintained. Finally, at each temperature, larger differences in germination percentages were recorded *in vitro* than *in vivo*. In contrast to this, the number of pollen tubes that reach the base of style and, hence, the success ratio, differed significantly among the male genotypes (Fig. 2b) ranging from 4.2 to 12.7 at 15°C and from 1.3 to 10.0 at 30°C.

Temperature affected significantly the adhesion of pollen grains to the stigmas (Table 2). The average number of adhered pollen grains was 677 at 15°C and 962 at 30°C. However a significant temperature-genotype interaction was recorded and the effect of temperature was significant for some genotypes. Higher adhesion at 30°C resulted in a higher number of germinated pollen grains, 375 at 30°C versus 293 at 15°C. The increase in the number of germinated pollen grains was not proportional to adhesion since the percentage of germination was reduced at the higher temperature, and this reduction was significant for only one donor. However, this higher number of germinated pollen grains did not result in higher numbers of pollen tubes at the base of the style, and a reduction in the number of pollen tubes was observed in the style at higher temperatures. Regarding the success ratio, its value was lower at the higher temperature for all but one genotype and averaged 7.2 at 15°C versus 3.9 at 30°C (Fig. 2b). Temperature-genotype interaction was also significant for this parameter indicating that the effect of temperature was genotype-dependent.

228

229 **Performance of one pollen donor across different female recipients**

230 To test if the female recipient had an effect on pollen performance, pollen from the same
231 pollen donor was used to pollinate eight different female recipients. The different female
232 genotypes showed a different capacity to adhere pollen grains and there were significant
233 differences among genotypes in pollen adhesion (Table 3), which ranged from 404 to 1114.
234 The stigmatic area varied between the female recipients from 0.56 mm² in ‘B. Provenza’ to
235 1.00 mm² in ‘Burlat’. This variation appears to contribute to the differences observed in
236 pollen adhesion since covariance analysis revealed a significant effect of the area (Table 3).
237 Different models of factor combinations with the covariable were examined and the results
238 proved a significant effect of the covariable and its interaction on the female recipient
239 genotypes. While the genotype of the female recipient itself explained part of the variation,
240 the significant area-female recipient interaction implies that the magnitude of the increase of
241 pollen adhesion with area varies with the female genotype.

242 As in the previous experiment, small differences were recorded in the percentage of pollen
243 germination (Fig. 3a). This resulted in a positive correlation between the number of adhered
244 and germinated pollen grains ($r = 0.73$, $n = 155$, $P < 0.001$) and to variable numbers of
245 germinated pollen grains that ranged from 128 to 406, depending on the female recipient. The
246 differences in the number of germinated pollen grains at the stigma were not taken over the
247 style in spite to the fact that clear and significant differences were recorded in the number of
248 pollen tubes reaching the base of the style and, hence, in the success ratio (Fig. 3b) depending
249 on the female genotype.

250 Temperature affected significantly pollen grain adhesion (Table 3), but the different female
251 genotypes responded differently to this effect and, while adhesion increased at 30°C for some
252 genotypes, it decreased for others. Indeed the recipient-temperature interaction was highly

significant indicating that the effect of temperature differed depending on the female recipient. Temperature did not affect the number of germinating pollen grains (Table 4) and affected significantly the percentage of germination in only one genotype (Fig. 3a). However, temperature had a reflection on the microgametophyte population that succeeded to reach the base of the style. The success ratio ranged from 0.3 to 30.4 at 15°C and from 0 to 10.9 at 30°C.

DISCUSSION

Pollen performance is affected by the pollen donor genotype

Pollen from the different pollen donor genotypes tested in this work behaved differently both *in vitro* and *in vivo*. *In vitro*, significant differences were recorded in pollen performance at each temperature regime among the different male genotypes. Several reasons can explain these differences in pollen performance. One explanation is that the differences are genetically determined (Sari-Gorla *et al.*, 1975; Pfahler *et al.*, 1997). An additional explanation is that the differences reflect the quality of the pollen sample. Indeed, pollen viability differs among species (Stone *et al.*, 1995), genotypes of the same species (Sari-Gorla *et al.*, 1975; Bookman, 1984; Pfahler *et al.*, 1997; Mckee & Richards, 1998; Hormaza & Herrero, 1999; Nikkanen *et al.*, 2000), or even among years on the same genotype (Nikkanen *et al.*, 2000). Finally, differences in pollen performance might be also due to differences in the nutritive status of the developing pollen grains, affected by environmental conditions (Young & Stanton, 1990; Jóhannsson & Stephenson, 1998) and, even inbreeding in the previous sporophytic generation (Jóhannsson *et al.*, 1998).

However, differences in pollen germination *in vitro* were not maintained *in vivo* and the percentage of germination *in vivo* differed little among pollen donors. Since pollen grain germination and initial pollen tube growth in the stigma occur in an autotrophic way (Herrero

& Dickinson, 1981) and initial pollen tube growth depends on the reserves stored in the pollen grain and on the pollen genotype (Jóhannsson & Stephenson, 1998; Stephenson *et al.*, 2003), it could be expected that pollen germination *in vivo* could resemble *in vitro* behavior. However, the results obtained here and in other species (Pfahler & Linskens, 1972; Stephenson & Bertin, 1983, Hormaza & Herrero, 1999) question the validity of the *in vitro* evaluation as a predictor of *in vivo* behavior. These differences could be explained considering that the stigma provides a rich germination environment equally suitable to the different male genotypes.

Whereas the stigma somehow appears to buffer genetic differences among pollen donor genotypes, pollen tube growth in the style was significantly different among male genotypes. Thus, the success ratio differed significantly among pollen donors on the same female recipient. Differences in pollen performance in the style had been previously recorded in other species and could represent a potential for sexual selection in plants through gametophytic selection (Snow & Spira, 1991; reviewed in Hormaza & Herrero, 1994).

The female recipient modulates pollen performance

While a genotypic component of pollen performance is clear, this component does not appear to perform in absolute terms, but in relation to the female recipient were the pollen tube grows. Thus, the same pollen donor showed a different performance in the different pistil genotypes. These observations confirm previous indications (Herrero & Hormaza, 1996) that a genetic pollen-style interaction could be superimposed over physical and nutritional differences among female genotypes. Other indications of the screening function of the style come from studies of gametophytic self-incompatibility (de Nettancourt, 2001), and from evidence of genetic interaction (Hormaza & Herrero, 1996). As a result of this interaction, better male-female combinations rather than better males could be occurring.

While the path of pollen tube growth along the style appears to be specially suited to favor this interaction and selection, the stigma appears to be well devised to capture and offer support for germination to an ample variety of individuals (Herrero, 1992; Herrero & Hormaza, 1996). Compared to *in vitro*, smaller differences in germination percentage were recorded *in vivo*, both among male genotypes on the same female recipient or among different female genotypes pollinated with the same pollen, as though if the stigma sets the basis for an equal opportunity starting line. This observation has been recorded in other cases, such as a delay in stigma maturation (Herrero, 1983; Murdy & Carter, 1987), a population effect where a minimum number of pollen grains is required for pollen germination to occur (Hormaza & Herrero, 1994), or the change in the pH of the stigma to allow pollen germination (Ganeshaiah & Uma Shanker, 1988). However, all stigmas did not appear to be equally successful in capturing pollen and some stigmas appear to gather over two times more pollen than others. This capacity appears to be related in part to the stigmatic area offered for pollen adhesion. Clear genotypic differences were recorded for this parameter and some genotypes had a stigmatic surface almost twice as large as that of others. A large stigmatic area may clearly contribute to enhance pollen capture and, consequently, pollen competition in the style. Nevertheless, the magnitude of the increase in pollen adhesion with area varied depending on the female genotype. Moreover, despite correction of variation by the covariable, the genotype of the female recipient still explains some variation in pollen adhesion. These results suggest that pollen adhesion may not be a merely physical process, and that other additional factors could potentially be operating.

Temperature affects pollen population census

Temperature had an effect on pollen performance, both on pollen adhesion and germination and on pollen tube growth. Furthermore, an interaction between the effect of temperature and

genotype (donor/recipient) has been recorded. At the stigma, pollen adhesion was affected by temperature since the higher temperature increased pollen adhesion uniformly when different pollen donors were used on a single female recipient. However, this response appears to be mediated by the female genotype, since a differential response was obtained when testing different female recipients. This differential response can be explained in terms of the stage of development of the stigma at anthesis. Differences in the degree of pistil development have been registered among flowers at anthesis (Rodrigo & Herrero, 2002) and temperature is known to accelerate stigma maturation and degeneration (Hedhly *et al.*, 2003). Thus, this acceleration of maturation or degeneration by temperature could increase or decrease pollen adhesion. However, the significant interaction between temperature and genotype implies that the magnitude of the effect of temperature depends on the genotype.

Pollen germination was reduced at 30°C both *in vitro* and *in vivo*, although differences were less conspicuous *in vivo*. A differential genotypic response was registered for this parameter. A decrease in pollen germination with increasing temperatures has been previously recorded in sweet cherry with different genotypes (Hedhly *et al.*, 2003, 2004) and could reflect the adaptation of this species to cool temperatures at blooming, since in other species optimum temperatures for pollen germination appear to reflect the adaptation of the species to the prevailing temperatures at their flowering time (Luza *et al.*, 1987; McKee & Richards, 1998).

Although a significant genotype-temperature interaction for pollen adhesion and germination at the stigma has been recorded, in this work genotype-temperature interaction was most conspicuous for pollen tube growth in the style. In general, the higher temperature reduced the number of pollen tubes that succeeded reach the base of the style, although the magnitude of the effect is genotype-dependent, since some genotypes were more severely affected than others. Previous work (Hedhly *et al.*, 2004) reported a similar effect for another sweet cherry cultivar, 'Sunburst'. However, pollen from 'Cristobalina', a sweet cherry cultivar adapted to

353 warm conditions, was favored under high temperatures compared to pollen from ‘Sunburst’.

354 The differences recorded here in relation to temperature among cultivars are not so

355 conspicuous. This may be related to the fact that the cultivars used in this work have a

356 narrower genetic variability for chilling requirements (900-1200 h) (Tabuenca, 1983) than the

357 two cultivars studied by Hedhly *et al.* (2004). A significant different competitive ability of

358 pollen to different temperatures, paralleling species tolerance to temperature, was initially

359 shown in *Lycopersicon* (Tanksley *et al.*, 1981). Later work in other species confirmed an

360 overlap in temperature response between the plant and its pollen (Luza *et al.*, 1987;

361 Kristjansdottir, 1990; Hormaza & Herrero, 1992, Mckee & Richards, 1998) and also a

362 genotype–environment interaction (Pasonen *et al.*, 2000, 2002), but further work is required

363 to elucidate the relationship of the response to temperature of the pollen donor plant and its

364 pollen performance. However, assuming fluctuating and heterogeneous temperature

365 conditions, the existence of a significant temperature-genotype interaction could prevent the

366 fixation of the genes affecting pollen tube growth.

367 The effect of environmental factors during pollen development may have significant

368 evolutionary consequences on pollen performance (Delph *et al.*, 1997). Clearly the nutritive

369 status of the plant may affect pollen performance and siring success (Young & Stanton, 1990;

370 Lau & Stephenson, 1993; Travers, 1999; Poulton *et al.*, 2001) but the fact that pollen

371 performance can respond to external environmental factors, such as temperature, provides an

372 opportunity for additional selection pressure occurring during the reproductive phase. This

373 would support the idea of gametophytic selection affecting the genetic composition of the

374 next sporophytic generation (Mulcahy, 1979) and provides a basis to understand the

375 successful adaptation of flowering plants to a wide range of environmental conditions.

376 The results obtained in this work show that pollen performance is affected by the male

377 genotype, the female recipient and that there were significant temperature-male and

temperature-female genotype interactions. These effects, while enabling a capacity of response to environmental changes, could maintain variability in pollen performance avoiding the fixation of the genes that control pollen tube growth rate. The maintenance of heritable variation in those genes is necessary for gametophytic and sexual selection to result in evolutionary changes.

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FIGURE LEGENDS

Fig. 1 Percentage of germinated pollen grains *in vitro* of 8 sweet cherry genotypes in controlled temperature chambers at 15°C and 30°C (mean + s.e.). Bars followed by the same letter within the same temperature are not statistically different (*Lsmeans option*, $P < 0.05$). * denotes significant differences between temperatures within the same genotype (*Lsmeans option*, $P < 0.0001$).

Fig. 2 Percentage of pollen germination and success ratio of eight pollen donors at the base of the style of one female recipient at constant temperatures of 15°C and 30°C (mean + s.e.). Bars followed by the same letter within the same temperature are not statistically different ($P < 0.05$). * denotes significant differences between temperatures within the same genotype ($P < 0.05$). *Duncan's Test for pollen germination and Lsmeans option for success ratio.*

Fig. 3 Percentage of germination and success ratio of one pollen donor genotype at the base of the style of several female recipients at constant temperatures of 15°C and 30°C (mean + s.e.). Bars followed by the same letter within the same temperature are not statistically different ($P < 0.05$). * denotes significant differences between temperatures within the same genotype ($P < 0.05$). *Duncan's Test for pollen germination and Lsmeans option for success ratio.*

Fig. 1

Fig. 2

Fig. 3

628 Table 1 Analysis of variance using GLM procedure for pollen germination *in vitro* of 9 pollen
629 genotypes.

Source	DF	Mean Square	F Value	Pr > F
Temperature	1	680.17	90.56	<.0001
Pollen Genotype	8	517.24	68.87	<.0001
Temperature*Genotype	8	25.28	3.37	0.0026
Error	69	7.51		

630

631 Table 2 Analysis of variance using GLM procedure for pollen adhesion (N°adh), germination
 632 (N°germ, %Germ) and success ratio (SR) *in vivo* in the experiment of one female recipient
 633 pollinated by eight male donors.

Source of variation	DF	N°Adh.		N°Germ.		%Germ.		S.R.	
		MS	Pr > F	MS	Pr > F	MS	Pr > F	MS	Pr > F
Temperature	1	2628027.29	<.0001	186005.03	<.0001	671.23	0.0003	1002.36	<.0001
Donor	7	811525.10	<.0001	86622.30	<.0001	218.82	0.0002	160.30	0.0034
Temperature*Donor	7	181842.82	0.0089	15769.76	0.2158	77.20	0.1413	126.63	0.0167
Error	141	63997.22		11348.36		48.20		49.28	

634

635 Table 3 Analysis of covariance using GLM procedure, taking the area as a covariable, for the
 636 number of adhered pollen grains in the experiment of eight female genotypes pollinated by
 637 one pollen donor genotype.

Source of Variation	DF	MS	Pr > F
Area	1	259081.02	0.0061
Area* Recipient	7	93840.36	0.0093
Temperature	1	254633.31	0.0066
Recipient	7	96625.82	0.0076
Temperature*Recipient	7	240403.18	<.0001
Error	131	33392.40	

638

639 Table 4 Analysis of variance using GLM procedure for pollen germination (N°Germ.,
 640 %Germ) and success ratio (SR) *in vivo* in the experiment of eight female genotypes pollinated
 641 by one pollen donor genotype.

Source	DF	N°Germ.		%Germ.		S.R.	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Temperature	1	34154.79	0.0524	488.84	0.0008	967.87	<.0001
Recipient	7	83401.07	<.0001	114.38	0.0109	1267.36	<.0001
Temperature*Recipient	7	44775.46	<.0001	55.27	0.2444	197.567	<.0001
Error	139	8924.55		41.82		28.41	

642