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Research article

The susceptibility and response of inbred and outbred *Mimulus guttatus* to infection by *Cucumber mosaic virus*

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Abstract. The deleterious effects of inbreeding have been well documented, but only recently have studies begun to explore the consequences of inbreeding for important ecological interactions. We examined the effects of inbreeding on the interaction between host and pathogen using the mixed-mating *Mimulus guttatus* (Scrophulariaceae) and *Cucumber mosaic virus* (CMV). Inbred (self) and outbred *M. guttatus* from two California populations (M5 and M13) were rub-inoculated with CMV and compared to sham-inoculated controls. Flower production by outbred plants in host population M5 showed little effect of the inoculation treatment, but inoculation reduced flower production of inbred plants by 12%, indicating that inbreeding reduces tolerance to CMV infection. This interaction fell short of significance, however. The effects of inbreeding and CMV inoculation on biomass in M5 varied significantly across the 15 families used in this experiment, indicating genetic variation in the effect of inbreeding on resistance or tolerance to CMV. CMV infection reduced biomass in host population M13, but there were no significant interactions between virus treatment and level of inbreeding for either flower production or biomass. Enzyme linked immunosorbent assay (ELISA) was used to detect CMV in host tissues. In both populations, mean ELISA absorbance values of inoculated plants were nearly identical for self and outcross hosts, indicating equal susceptibility to CMV. In outbred plants of population M5, flower production did not change with increasing ELISA absorbance, but in inbred plants it declined, indicating reduced tolerance to CMV infection. The results from this study suggest that pathogens may become increasingly detrimental as host populations become more inbred.

Key words: *Cucumber mosaic virus*, CMV, disease, inbreeding, *Mimulus guttatus*, pathogen, resistance, tolerance

Introduction

Inbreeding reduces genetic variation within offspring. This is typically associated with a decline in fitness (inbreeding depression) due either to the expression of recessive or partially recessive deleterious alleles or to the loss of heterozygosity at loci segregating alleles that interact in an overdominant manner (Falconer, 1981; Lynch and Walsh, 1998). The expression of inbreeding depression is often influenced by environmental factors (Dudash, 1990; Crnokrak and Roff, 1999; Keller and Waller, 2002). Only a few studies

have examined the effect of inbreeding on important interspecific interactions, including those with competitors (Cheptou *et al*., 2000), herbivores (Núñez-Farfán *et al*., 1996; Carr and Eubanks, 2002), and pathogens (Ouborg *et al*., 2000). This study examines the effects of inbreeding on the response of *Mimulus guttatus* (Scrophulariaceae) to infection by *Cucumber mosaic virus* (CMV).

Interactions between inbred plants and natural enemies are likely to be common. In insect pollinated plants, selfing and mixed-mating (i.e., partial self-fertilization) may be the rule rather than the exception (Vogler and Kalisz, 2001), and plant populations should be generally prone to inbreeding because of local population structure (Levin, 1988). Because variation in disease resistance in many natural plant populations is genetically based (Parker, 1985; Burdon, 1987; De Nooij and van Damme, 1988; Alexander, 1989; Jarosz and Burdon, 1991; Bevan *et al*., 1993; Davelos *et al*., 1996) understanding the effect of inbreeding on resistance is essential in understanding the host-pathogen interaction. If inbreeding alters susceptibility or resistance, the evolutionary response of mixed-mating host–populations to pathogens could be altered (Kelly, 1999a, b), and metapopulation dynamics of host and pathogens could be altered (Antonovics *et al*., 1994). The effect of pathogens on progeny derived from self-pollinations could influence the magnitude of inbreeding depression**,** and hence**,** mating-system evolution (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987, 1990; Charlesworth *et al*., 1990; Cheptou and Mathias, 2001).

Ouborg *et al*. (2000) found that inbreeding in *Silene alba*, on average, decreased the infection rate by the anther smut fungus, *Microbotryum violaceum*, by 28%. However, the response to inbreeding varied significantly among study populations and among inbred lines within populations, with many populations and lines showing declines in resistance with increased level of inbreeding. The investigators suggested that predicting population responses to inbreeding and pathogens might be difficult, especially since inbreeding could affect not only resistance but also, for example, the attractiveness of hosts to potential disease vectors.

The effect of inbreeding on resistance and tolerance to viruses has not been examined, but we suggest that inbreeding may produce both direct and indirect effects. Inbreeding could have direct effects on the response to viral infection if genes involved in plant defenses against viruses are segregating deleterious alleles or if alleles act in an overdominant fashion. If so, we expect resistance to erode with inbreeding. Inbreeding could also indirectly alter apparent resistance and tolerance. The overall physiological state of the host has a strong influence on the virus. A healthy, vigorous plant may be a better host for the virus because of the virus' dependence on the host for production of viral gene products and cell–cell and phloem-dependent movement (Hull, 2002). If in-

breeding reduces overall vigor, as is commonly seen (Charlesworth and Charlesworth, 1987), inbred plants may actually be poor hosts for the virus.

The *M. guttatus* species complex has been the subject of numerous studies of inbreeding depression and mating-system evolution (Dole and Ritland, 1993; Willis, 1993a, b, 1999; Carr and Dudash, 1995, 1996, 1997; Dudash *et al.*, 1997). Carr and Eubanks (2002) demonstrated that inbreeding altered host plant quality and tolerance to damage inflicted by the spittlebug, *Philaenus spumarius*, but as in the *Silene-Microbotryum* system (Ouborg *et al.*, 2000), the effects varied between populations. While tolerance to spittlebug feeding was unaltered by inbreeding in one population, tolerance was reduced in inbred plants of another population. In that population, outbred plants were virtually unaffected by spittlebugs, but in inbred plant spittlebugs reduced flower production by 13% and biomass by 30%. The effects of inbreeding on host plant quality also differed between populations. Spittlebugs emerged 16% larger in one population, indicating an increase in host plant quality with inbreeding, while spittlebugs took 10% longer to mature on inbred plants in the other population, indicating a decrease in host plant quality.

In this study, we have addressed whether or not inbreeding alters the response of *M. guttatus* to infection by CMV. We chose to study the interaction of a plant virus with *M. guttatus* because of the intimate relationship between a virus and its host. This relationship is likely to be dramatically affected by changes in host gene expression. This study is the first to report the responses of inbred and outbred progeny to virus infection.

Materials and methods

Study organisms

Mimulus guttatus DC (Scrophulariaceae) populations occur throughout western North America from Mexico to Alaska, occupying a variety of moist, open habitats. Estimated mating systems from different populations vary from about 75% selfing ($t = 0.25$) to complete outcrossing ($t = 1.0$), averaging about $t \approx 0.60$ (Ritland and Ritland, 1989; Ritland, 1990; Dudash and Ritland, 1991; Willis, 1993a). *Mimulus guttatus* typically produces a pair of large (~20–30 mm in width), perfect, yellow, zygomorphic flowers at each flowering node and may produce as many as 100 or more flowers during a season. Populations in habitats that are moist year-round are commonly perennial, but in seasonally dry areas populations tend to be annual (Dole, 1992).

Field-collected *M. guttatus* seed for this experiment came from two annual populations separated by 160 km, one (M5) in Santa Clara County, CA, USA (37°17′ N, 122°09′ W) and the other (M13) in Napa County, CA (38°33′ N,

122°22′ W). Outcrossing rates have not been estimated for either population. These populations and families are the same as those used by Carr and Eubanks (2002), although only 13 of the original 15 families were available for M13.

There is little information on naturally occurring viruses in *M. guttatus*. CMV was chosen for this study because it has one of the broadest host ranges among viruses that infect plants (Palukaitis *et al.*, 1992). While many important agricultural crop plants are among the more than 800 plant species known to be hosts for CMV, so too are many non-crop species that are thought to harbor the virus between cropping seasons and serve as sources of inoculum (Tomlinson *et al.*, 1970). Plants within the Scrophulariaceae have been shown to be susceptible to infection by CMV (Brunt *et al.*, 1996). The ubiquitous nature of CMV results, in part, from its ability to be transmitted by at least 75 species of aphid in a nonpersistent manner (Palukaitis *et al.*, 1992). Aphids can acquire the virus on their stylets within seconds or minutes of feeding on an infected plant, and transmit the virus to a new host with no latent period.

In a susceptible CMV–host interaction, CMV is typically introduced into a leaf epidermal cell by an aphid vector, or experimentally, through a wound created by the mechanical inoculation process. The CMV genome consists of three single-stranded messenger sense RNA molecules each packaged in its own particle by coat protein. Upon entering a living cell, the infection process is initiated by production of viral-encoded proteins and replication of the viral RNA molecules. All three RNA molecules are required for a complete infection, i.e., replication, cell-to-cell and phloem-dependent movement leading to systemic infection. During the cell-to-cell movement process, CMV will move into vascular tissues, particularly phloem, through which it is rapidly transported to tissues that act as sinks for photoassimilates. This phloem-dependent movement process typically results in movement of CMV into the roots and young developing tissues and reproductive tissues (Palukaitis *et al.*, 1992). This process of systemic invasion by CMV typically results in the dramatic symptoms observed in most hosts, such as chlorosis, mosaic, blistering and deformation of leaves. While the severity of these symptoms may vary with host, virus strain and environmental conditions, a general stunting of plant growth resulting from a lack of extension of internodes is extremely common. For many plants, systemic infection by CMV simulates or perhaps triggers early senescence.

The KM isolate of CMV was used throughout this study (Guerini and Murphy, 1999). The virus was propagated in an aphid-free, temperature controlled greenhouse (28 °C day/21 °C night) at the Auburn University Plant Science Greenhouse Complex in Auburn, Alabama, USA. This CMV culture has been maintained for several years by mechanical passage in *Nicotiana tabacum* 'Kentucky 14' or *Capsicum annuum* 'Early Calwonder'.

Crossing design

In the fall of 1998, field-collected seed from both *M. guttatus* populations was sown in a pollinator-free greenhouse at the Blandy Experimental Farm (BEF) in Boyce, VA, USA. Population M5 was represented by 15 maternal families, and population M13 was represented by 13 maternal families. One individual grown from each of these families served as the maternal plant for the production of both self and outcross seed. We emasculated all flowers in bud prior to hand-pollination (both self- and cross-pollinations). To produce outcross seed, we randomly paired individuals from the same population, applying pollen directly from the anthers of the pollen donor to the receptive stigma of the recipient. Each family served only once as a pollen donor for outcross seed. Self-fertilized seed was produced by applying pollen from another flower on the same plant to the receptive stigma of a newly opened, previously emasculated flower (geitonogamous pollination).

Plants were grown in 70×70 mm pots filled with professional growing medium (Wetsel, Harrisonburg, VA, USA). Pots were arranged 20 to a tray and bottom watered. No fertilizer was used. Sodium vapor lights extended the photoperiod to 18 h as needed after the seedlings were transplanted.

Experimental design

Fifty self and outcross seeds from each maternal family in each of the two populations were sown on 26 September 2000 in the greenhouse at BEF. On 13 October, 10 randomly selected seedlings from each cross from each maternal family were transplanted into separate pots. Half of these seedlings were randomly designated as control plants and the other half were designated for inoculation with CMV. Plants were arranged in a randomized complete block design with a control and experimental plant from each cross of each maternal family contained within each of five blocks (A–E). Growing conditions were identical to those described above.

On 26 October 2000**,** plants were inoculated with CMV. CMV inoculum consisted of systemically infected Kentucky 14 leaf tissue ground in 50 mM potassium phosphate buffer, pH 7.5. All materials (e.g., mortar, pestle and buffer) were chilled to 4 °C prior to inoculation and were maintained on ice during inoculation procedures. Plants were lightly dusted with the abrasive carborundum prior to inoculation. CMV was inoculated onto the two to three oldest leaves of plants by rub-inoculation using an inoculum-saturated cotton swab. Plants representing the non-virus inoculated control treatment were subjected to a similar inoculation treatment but without addition of virus to the buffer.

Plants were monitored for CMV infection visually and by detection of virus in leaves. For visual assessments, plants were routinely examined for the occurrence of CMV-induced symptoms such as chlorosis and mosaic patterns on leaves, leaf deformation and overall plant stunting resulting from shortened internodes. To determine the presence of CMV in leaves, one leaf was collected from each of the first two (oldest) nodes of each plant. The samples were placed in individual whirl bags and shipped to Auburn University for analysis. CMV was detected using indirect enzyme-linked immunosorbent assay (ELISA), a serological assay that detects the viral coat protein and is used as a measure of virus accumulation. Each sample was processed for analysis by grinding in the whirl bag in 1 ml of 50 mM carbonate buffer, pH 9.6, using a BioReba homogenizing device (BioReba Ag, Switzerland). All subsequent steps were as described by Guerini and Murphy (1999).

The effect of CMV on plant fitness was measured by censusing total flower production and aboveground biomass. Plants in blocks A and B were scored on 11 December, C and D on 12 December, and E on 13 December. Once the number of flowers had been counted, plants were harvested at soil level and dried to a constant weight. Each plant was weighed to the nearest 0.01 g using an Ohaus top-loading balance.

Data analysis

Because the effects of inbreeding and outbreeding are expected to be influenced by the unique history and ecology of each population, we examined the effect of inbreeding in M5 and M13 in separate analyses. Total flower production was square-root transformed, and plant aboveground biomass was log-transformed. Bartlett's test of homogeneity of variance revealed no significant heterogeneity among treatment variances. Residuals from ANOVAs matched well with normal probability plots. Both of these dependent variables were analyzed by a similar mixed-model ANOVA consisting of the following effects: block (random), pollination treatment (fixed), virus treatment (fixed), family (random), pollination × virus treatment (fixed), and the random two- and three-way interactions between pollination treatment, virus treatment, and family.

Inbreeding depression (δ) for each family, i, was estimated by

$$\delta_i = (w_{Oi} - w_{Si})/w_{Oi} \quad (1)$$

where w_O and w_S are the fitness trait (flower number or biomass) means for outcross and self-offspring, respectively. In the rare cases where the mean fitness of inbred plants exceeded that of outbred (15 of 120 calculations in M5 and 5 of 104 calculations in M13), the inbred mean was substituted into the denominator following Ågren and Schemske (1993). Mean inbreeding de-

pression for each population was calculated as the mean of all family inbreeding depression estimates. Inbreeding depression for control and inoculated plants was compared by paired *t*-tests.

To examine the effects of inbreeding on virus infection, ELISA absorbance values were analyzed separately for each population using a mixed-model ANOVA. The models included the following sources of variation: block (random), pollination treatment (fixed), family (random), pollination treatment × family (random).

To explore the effect of virus infection (based on ELISA absorbance) on plant fitness traits (flower production and biomass), a second analysis was performed using only the inoculated plants. We applied a heterogeneity of slopes model that included block and family as random effects, pollination treatment as a fixed effect, and ELISA absorbance as a covariate. Pairwise and three-way interactions were also included. Interactions between pollination treatment and ELISA absorbance would indicate that plants derived from selfing respond differently to increasing levels of infection than do plants derived from outcrossing.

All analyses were performed with SAS (version 8e) Proc GLM. The significance of all fixed effects in the mixed models ANOVAs were tested with appropriate random interaction mean squares as error terms following Sokal and Rohlf (1995). Custom hypothesis tests were created with the Test statement.

Results

Inbreeding and viral pathology

Symptoms of CMV infection were conspicuous on nearly all inoculated plants. Chlorosis and leaf curling were observed in 70%. Thirty-six percent of inoculated plants had severe flower deformation, including reduced size, a pale corolla, and petals that failed to fuse into the characteristic zygomorphic corolla.

The two populations differed in the effects inbreeding had on CMV pathology. In population M5, the virus had no detrimental effect on flower production in outcross plants, but self-plants infected by CMV produced 12% fewer flowers than self-controls (Fig. 1a). The interaction between pollination treatment and virus treatment fell short of significance, however (Table 1a). Both selfing and virus infection significantly reduced aboveground biomass in population M5, but the two treatments did not interact significantly (Table 1b). CMV reduced biomass by approximately 50 mg in both self and outcross plants (Fig. 1b). However, there was a significant three-way

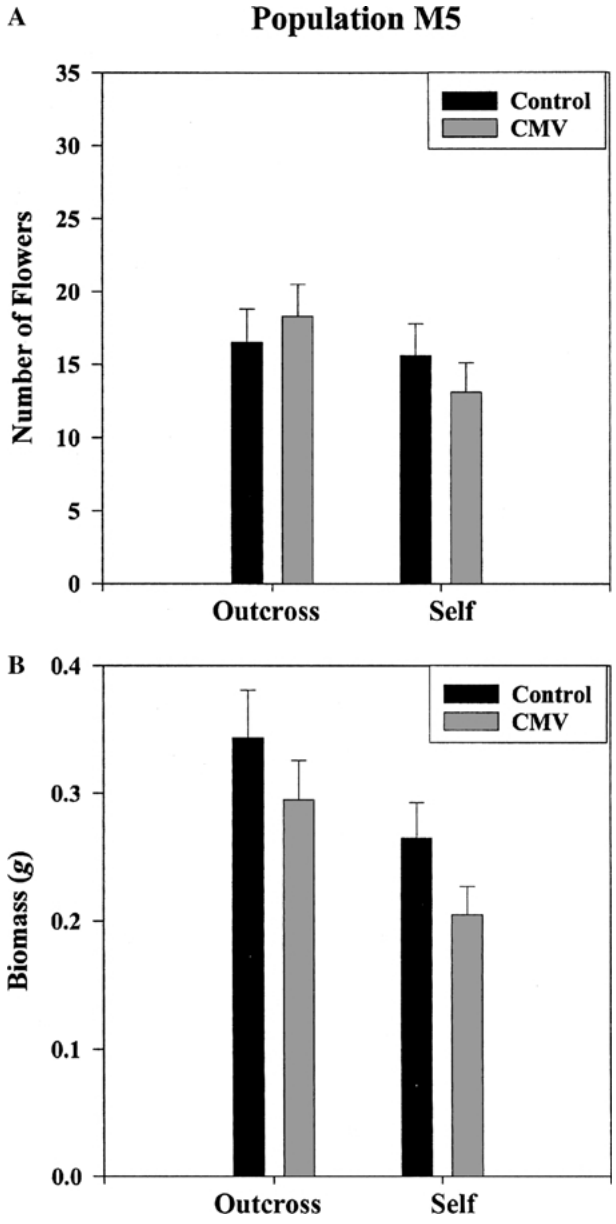

Figure 1. Flower production (a) and biomass (b) for self and outcross *Mimulus guttatus* from population M5. Plants were either inoculated with CMV or sham inoculated (control). Error bars indicate 95% confidence limits. Pollination treatments differ significantly from one another for both fitness traits, but the virus treatment is significant for only biomass. The pollination × virus interaction is not significant for either trait.

Table 1. Mixed-model analysis of variance for dependent variables (a) flower production and (b) biomass for host population M5

Source	df	MS	*F*	*p*
(a) Flower production				
Block	4	1.398	1.58	0.1791
Pollination	1	15.838	6.73	0.0212
Virus	1	0.016	0.02	0.8905
Family	14	4.034	4.57	<0.0001
Poll. × Virus	1	3.423	2.91	0.1098
Poll. × Family	14	2.352	2.67	0.0012
Virus × Family	14	0.815	0.92	0.5340
Poll. × Virus × Fam	14	1.174	1.33	0.1896
Error	234	0.882		
(b) Biomass				
Block	4	0.321	1.89	0.1135
Pollination	1	7.285	15.57	0.0015
Virus	1	3.125	21.14	0.0004
Family	14	1.077	6.32	<0.0001
Poll. × Virus	1	0.189	0.62	0.4443
Poll. × Family	14	0.468	2.75	0.0009
Virus × Family	14	0.148	0.87	0.5941
Poll. × Virus × Fam	14	0.306	1.80	0.0399
Error	234	0.170		

Flower production was square-root transformed, and biomass was log-transformed. Pollination treatment (self or outcross), virus treatment (inoculated or uninoculated), and their interaction are fixed effects. Error terms for effects followed Sokal and Rohlf (1995).

interaction among family, pollination treatment and virus treatment (Table 1b). In nine families, self-plants were more severely impacted by CMV infection, but in the remaining six, outcross plants were more severely affected by the virus (Fig. 2). These analyses suggest genetic variation for the response to inbreeding and virus infection in M5 but not necessarily a population level trend in the effect of inbreeding on tolerance to virus infection.

In host population M13, selfing significantly reduced flower production, but CMV infection had little effect on flower production in either self or outcross plants (Table 2a, Fig. 3a). As in population M5, both selfing and CMV infection reduced biomass in M13 (Table 2b, Fig. 3b). The treatments did not interact, however, with virus infection reducing biomass by 50–60 mg in both self and outcross plants. The interaction between family and pollination treatment was significant in population M13 for biomass, but there were no significant interactions involving the virus treatment (Table 2b). These analyses suggest that population M13 shows variation in the response to inbreeding, but inbreeding does not alter response to CMV.

Our estimates of inbreeding depression, δ, for inoculated plants were consistently higher than estimates in controls, but the difference in inbreeding

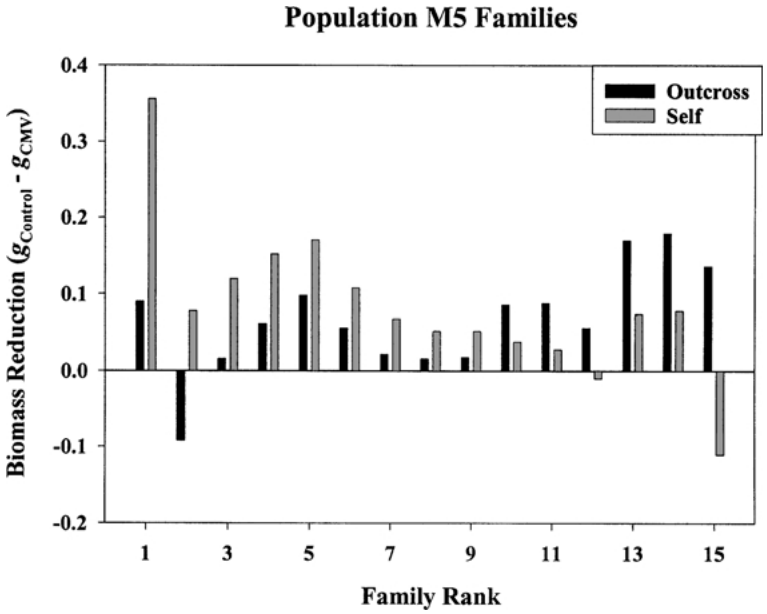

Figure 2. Differences between mean control and mean CMV infected *Mimulus guttatus* for 15 maternal families in population M5. The pollination × virus × family interaction is significant.

depression approached significance only in the comparison of inoculated and control flower production in population M5 ($t = 2.13$, $p = 0.052$). In M5, inbreeding depression based on flower production increased from 9% (±10.0) in controls to 27% (±5.9) in inoculated plants and from 19% (±8.2) in controls to 28% (±6.0) in inoculated plants based on biomass. In population M13, inbreeding depression showed a modest increase from 28% (±6.4) to 32% (±5.2) and from 23% (±7.1) to 31% (±6.6) based on flower production and biomass, respectively.

Inbreeding and virus infection

The presence of CMV in inoculated plants was confirmed by ELISA. A 'background' reaction to host proteins is not unusual (Murphy *et al.*, 1999), and a fairly strong background reaction occurred with M5 plants. However, positive reactions among CMV-inoculated plants were clearly and significantly greater than the control values (mean ± SE = 0.57 ± 0.02 vs. 0.38 ± 0.02 for M5 and 0.52 ± 0.01 vs. 0.16 ± 0.01 for M13). Control plants showed no symptoms of CMV infection, and more sensitive testing in another experiment suggested that contamination was not a problem (J.F. Murphy *et al.*, unpublished data). Among the inoculated plants, we observed no difference between self and outcross treatments in mean ELISA absorbance in either M5

Table 2. Mixed-model analysis of variance for dependent variables (a) flower production and (b) biomass for host population M13

Source	df	MS	*F*	*p*
(a) Flower production				
Block	4	6.438	6.91	<0.0001
Pollination	1	41.343	28.79	0.0002
Virus	1	0.000	0.00	0.9950
Family	12	3.863	4.15	<0.0001
Poll. × Virus	1	0.294	0.44	0.5204
Poll. × Fam	12	1.436	1.54	0.1144
Virus × Fam	12	0.446	0.48	0.9249
Poll × Virus × Fam	12	0.671	0.72	0.7300
Error	159	0.931		
(b) Biomass				
Block	4	1.336	6.96	<0.0001
Pollination	1	6.112	17.98	0.0011
Virus	1	3.099	38.63	<0.0001
Family	12	1.607	4.88	0.0051
Poll. × Virus	1	0.309	2.23	0.1615
Poll. × Family	12	0.340	1.77	0.0438
Virus × Family	12	0.080	0.42	0.9548
Poll. × Virus × Fam	12	0.139	0.72	0.7279
Error	158	0.192		

Flower production was square-root transformed, and biomass was log-transformed. Pollination treatment (self or outcross), virus treatment (inoculated or uninoculated), and their interaction are fixed effects. Error terms for effects followed Sokal and Rohlf (1995).

(0.57 ± 0.02 for self vs. 0.57 ± 0.02 for outcross) or M13 (0.54 ± 0.04 for self vs. 0.50 ± 0.04 for outcross), suggesting that inbred and outbred plants are equally susceptible to CMV infection by rub inoculation (Table 3). In population M5, however, there was significant variation in ELISA absorbance values among maternal families, suggesting possible genetic variation in susceptibility or accumulation (Table 3).

Using ELISA absorbance as a covariate in a heterogeneity of slopes model, CMV appeared to have different effects on flower production by inbred and outbred M5 plants, as indicated by a significant interaction between ELISA absorbance and pollination treatment (Table 4a). In examining inoculated outcross plants alone, flower production does not change across the range of ELISA absorbance (Fig. 4a). In contrast, flower production in self-plants declined with increasing ELISA absorbance (Fig. 4b). Self-plants appear less tolerant of CMV infection. A similar effect on flower production was seen in population M13, but a significant ELISA × family interaction and a three-way interaction among families, pollination treatment, and ELISA absorbance suggests that there is considerable variation in this response (Table 4b).

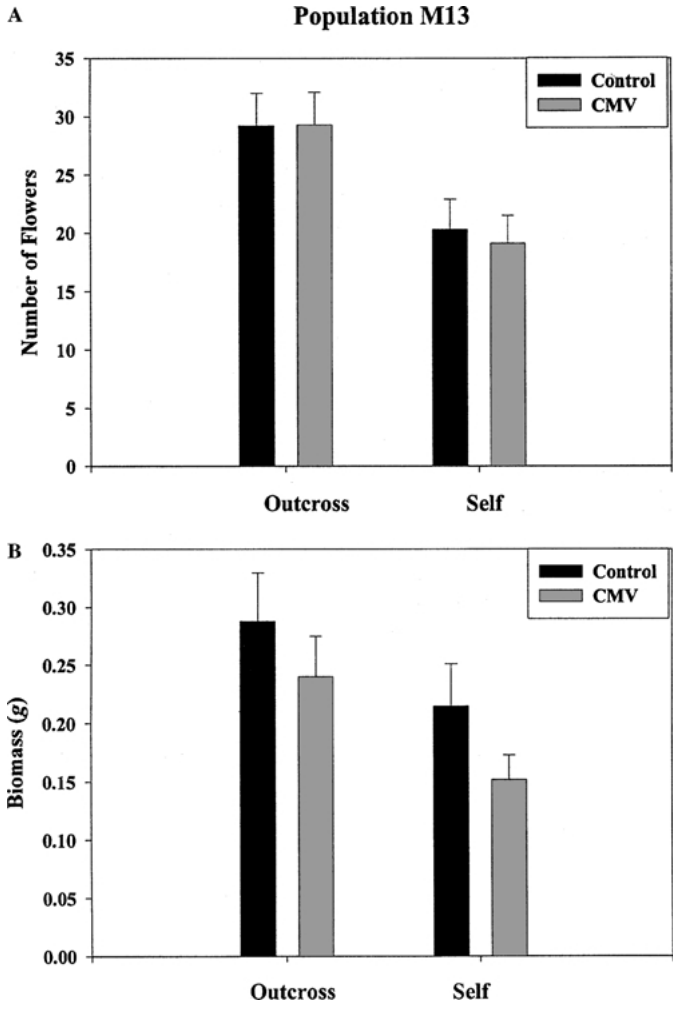

Figure 3. Flower production (a) and biomass (b) for self and outcross *Mimulus guttatus* from population M13. Plants were either inoculated with CMV or sham inoculated (control). Error bars indicate 95% confidence limits. Pollination treatments differ significantly from one another for both fitness traits, but the virus treatment is significant for only biomass. The pollination × virus interaction is not significant for either trait.

Discussion

Our results on flower production in population M5 suggest that inbreeding can reduce host tolerance to viral infection. While CMV infection slightly increased flower production in outbred *M. guttatus* from population M5, inbred plants from M5 showed a 12% reduction in the number of flowers produced, though this only approached significance ($p = 0.1098$). The increase in flower produc-

Table 3. Mixed-model analysis of variance comparing log-transformed ELISA absorbance from self and outcross plants inoculated with CMV in host populations (a) M5 and (b) M13

Source	df	MS	*F*	*p*
(a) Population M5 ELISA absorbance				
Block	4	2.168	18.082	<0.0001
Pollination	1	0.006	0.103	0.7525
Family	14	0.362	3.018	0.0005
Poll. × Family	14	0.053	0.443	0.9567
Error	115	0.120		
(b) Population M13 ELISA absorbance				
Block	4	3.143	7.311	<0.0001
Pollination	1	0.135	0.615	0.2103
Family	12	0.253	0.588	0.3622
Poll. × Family	12	0.206	0.479	0.9218
Error	81	0.430		

Pollination treatment (self or outcross) is a fixed effect. Error terms for effects followed Sokal and Rohlf (1995).

Table 4. Heterogeneity of slopes model examining changes in flower production with changes in ELISA absorbance in host populations (a) M5 and (b) M13

Source	df	MS	*F*	*p*
(a) Population M5				
Block	4	0.839	0.87	0.3375
Family	14	0.614	0.64	0.0047
Pollination	1	2.560	4.51	0.0520
Elisa	1	0.018	0.02	0.8916
Fam × Poll.	14	0.568	0.59	0.8656
Fam × ELISA	14	0.628	0.65	0.8121
Poll. × ELISA	1	5.116	8.46	0.0114
Fam × Poll. × ELISA	14	0.605	0.63	0.8338
Error	85	0.961		
(b) Population M13				
Block	4	2.236	2.61	0.0451
Family	12	2.524	2.95	0.0031
Pollination	1	2.738	1.42	0.2591
Elisa	1	8.052	9.41	0.0033
Family × Poll.	12	1.934	2.26	0.0236
Family × ELISA	12	2.013	2.35	0.0161
Poll. × ELISA	1	4.705	2.62	0.1341
Fam × Poll. × ELISA	12	1.799	2.10	0.0355
Error	55	0.856		

tion for outbred M5 plants may have resulted from a more rapid onset of senescence due to the reduced growth compared with uninoculated control plants. In contrast, inbred M5 plants may have been so severely affected by CMV that reproductive processes may have been shut down. The lower tolerance of inbred

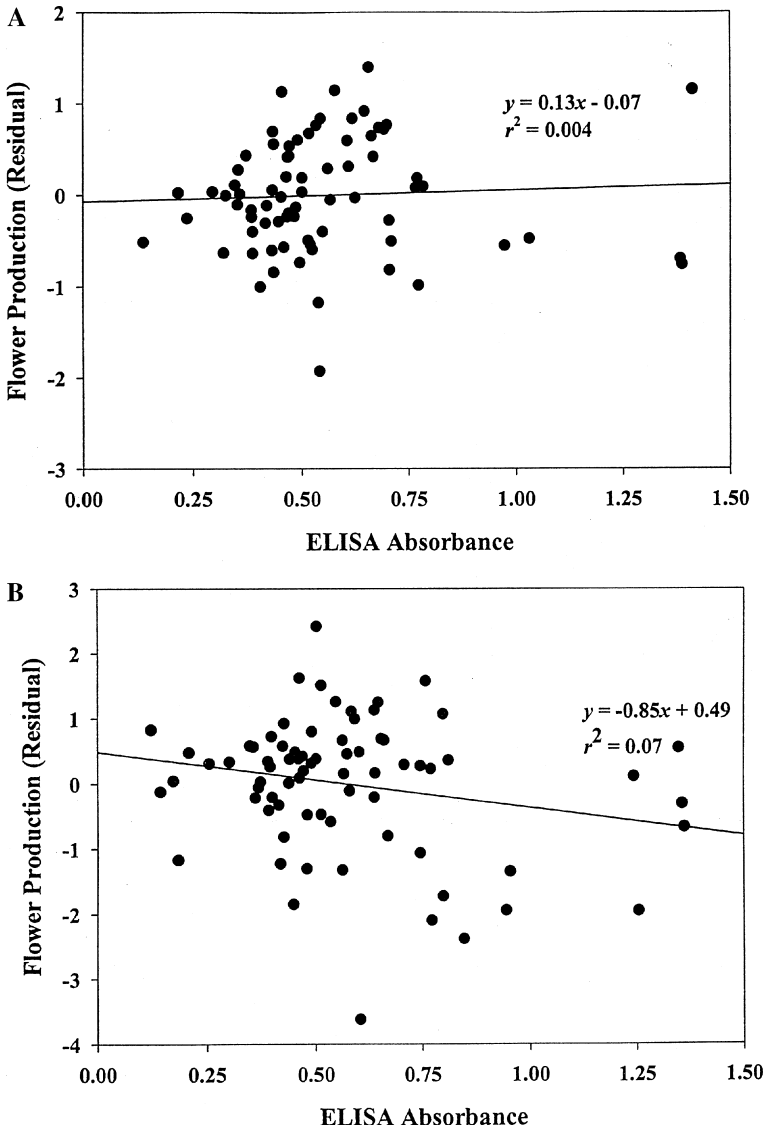

Figure 4. The response of inoculated outbred (a) and inbred (b) plants from host population M5 to variation in ELISA absorbance. The response variable is flower production after the removal of variation due to block and family. Slopes differ significantly from one another (pollination × ELISA $F_{1,14} = 8.46$, $p = 0.0114$).

plants was more in evidence when infection level indicated by ELISA absorbance was considered. While flower production in outbred M5 plants remained consistent across the range of infection observed in inoculated plants, flower production declined in inbred plants with increasing infection level.

Differences in CMV disease severity may result from differences in levels of susceptibility, virus accumulation, the rate and extent of movement throughout the plant, or combinations thereof (Hull, 2002). Significant variation among M5 families in ELISA absorbance indicates that host plant genotype may influence susceptibility or accumulation. The fact that mean ELISA absorbance was nearly identical in self and outcross plants suggests that the more pronounced effect of CMV on inbred plants was not due to a difference in susceptibility to CMV. Rather, inbred and outbred plants did not respond to infection in the same way. Since both cell-to-cell and phloem-dependent movement require compatible interactions between viral encoded proteins and host factors (e.g., microtubules and plasmodesmata; Heinlein *et al*., 1995; Nelson and van Bel, 1998), genetic changes associated with inbreeding and genetic variation among the different families, while perhaps subtle in degree, can have a direct effect on CMV infection.

Our results suggest that inbreeding need not always alter the response to viral infection or need not always result in decreased tolerance or resistance. Flower production in both inbred and outbred M13 plants was unaffected by CMV infection, and biomass was reduced by CMV to a similar extent in inbred and outbred plants. Variation in the effect of inbreeding was also evident within populations. In six M5 families the reduction in biomass in infected self-plants was actually smaller than that in infected outbred plants. Inbred and outbred families in M13 varied significantly in their response to increasing virus accumulation (as indicated by ELISA absorbance). These within population differences may represent genetic variation, though maternal effects are not excluded. Population and family differences in response to inbreeding are commonly found in studies examining fitness traits in plants (e.g., Ågren and Schemske, 1993; Dudash *et al*., 1997) and animals (Pray and Goodnight, 1995). These differences may represent differences in the history of inbreeding (Campbell, 1986; Uyenoyama and Waller, 1991) or may result from random differences among lineages in the accumulation of deleterious mutations (Schultz and Willis, 1995). The variation among inbreeding lines is evolutionarily significant in that it represents the opportunity for selection to improve or maintain resistance or tolerance in an inbreeding population. Similarly, differences among populations may be due to differences in selection regimes.

In nature CMV is vectored by aphids, while in our experiment plants were mechanically inoculated with CMV by rub-inoculation. Whether inbred or outbred plants might serve as better sources of CMV inoculum or may be more efficiently inoculated by aphid vectors was not addressed. Ouborg *et al*. (2000) found that inbreeding reduced a number of floral traits that have been shown to be important in pollinator attraction and concluded that inbred plants may receive fewer fungal spores from the pollinator-vectored *Microbotryum*. Since inbreeding appears to affect plant–herbivore interactions (Carr and Eubanks,

2002), the effect of inbreeding on the dynamics of the host–pathogen relationship cannot be fully understood until its effect on transmission is examined.

In contrast to our study, Ouborg *et al.* (2000) found that inbreeding had a tendency to decrease susceptibility to infection in the *Silene-Microbotryum* system. Similar to our findings, however, host populations and lineages varied in their response to inbreeding and infection. Results from a study in an insect–parasite system also have shown variable responses. Stevens *et al.* (1997) found that inbred lineages of the flour beetle, *Tribolium castaneum,* varied in their susceptibility to tapeworm infection. Although most inbred lineages showed no change in susceptibility relative to the base (outbred) population, four of the five lineages that differed significantly from the base population showed decreased susceptibility. The increased resistance to infection in response to inbreeding found in these studies suggests that heterozygosity *per se* does not confer greater resistance in these systems. The types of allelic interactions must be dominance or partial dominance and not overdominance, quite possibly with alleles conferring greater resistance being recessive or partially recessive.

Inbreeding in vertebrates seems to produce a more consistent decline in resistance or tolerance to parasites and pathogens. Coltman *et al.* (1999) found that inbred Soay sheep, *Ovis aries*, had higher parasite loads and higher mortality than outbred sheep. O'Brien and Evermann (1988) examined 13 cases in which mammal populations were known to have experienced recent population bottlenecks, asserting that the loss of genetic variation frequently led to post-bottleneck infectious disease epidemics. The consistent decline in resistance with inbreeding in vertebrates may due to the importance of variation at the major histocompatibility complex (MHC) in the vertebrate immune response (Hedrick, 1994), though direct causation has yet to be established. The observation of improved resistance or tolerance in plants here and elsewhere (Ouborg *et al.*, 2000) suggests a very different genetic system, one in which variation in resistance is most likely due to deleterious recessive alleles. Such should be the case in gene-for-gene systems in which resistance is most commonly conveyed by dominant alleles (Crute, 1994).

Carr and Eubanks (2002) examined the effects of inbreeding on interactions with spittlebug (*Philaenus spumarius*) herbivores for plants of these same *Mimulus* populations. Again differences were observed in the response of each population to inbreeding, but in this case it was host population M13 that showed reduced tolerance to herbivory with inbreeding. There was virtually no impact of the spittlebugs on outcrossed plants in M13, but flower production in self-plants fed on by spittlebugs decreased by 13% and biomass decreased by 30% relative to controls. The populations also differed in the effect of inbreeding on host plant quality. Spittlebugs raised on inbred plants in population M5 were 16% larger than those developing on outbred plants, suggesting an increase in host plant quality with inbreeding. In population

M13, spittlebug nymphs took 10% longer to mature on inbred plants, suggesting a decrease in host plant quality. Together these studies suggest that multiple genetic systems are involved with plant interactions with natural enemies, and that there may be potential tradeoffs in resistance to some herbivores and pathogens.

Our data here and in a previous study (Carr and Eubanks, 2002) suggest that interactions with natural enemies may increase inbreeding depression. Pathogens and herbivores may become increasingly debilitating as host populations become more inbred. This could alter selection for resistance (Kelly, 1999a, b), mating system evolution (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1990; Charlesworth *et al*., 1990), or the extinction rate of small populations (Saccheri *et al*., 1998).

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