



High gene flow levels lead to gamete wastage in a desert spider system

Susan E. Riechert¹, Frederick D. Singer² & Thomas C. Jones¹

¹Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville TN 37996-1610, USA (Phone: (865) 974-6187; Fax: 865 (974-3067); E-mail: sriecher@utk.edu); ²Department of Biology, University of Radford, Radford VA 24142, USA

Key words: *Agelenopsis aperta*, behavioral genetics, behavioral phenotype, desert, gamete wastage, gene flow, introgression, postzygotic barriers, prezygotic barriers

Abstract

Field censuses, breeding experiments, and a quantitative model are used to obtain insight into the extent and consequences of genetic mixing between locally adapted populations of a desert spider. Typically, 9% of the matings of desert riparian spiders (non-aggressive phenotype) in native habitat involve an arid-land partner (aggressive phenotype). Mating was found to be random with respect to behavioral phenotype, but linearly related to both the rate of immigration and survival of immigrants from surrounding arid habitats in the riparian area. Genetic mixing between riparian and arid-land spiders produces offspring that exhibit lower rates of survival in riparian habitat. Two extreme behavioral phenotypes were also observed in the field studies: approximately 5% of the female spiders attacked all males they encountered while another 22% ran from all potential mates. Punnett square analyses of the potential genotypes produced by introgression between arid- and riparian-adapted spiders indicate that these extreme phenotypes appear in F2 generation hybrids and backcrosses. Because there is a costly wastage of gametes in the case of mixed phenotype mating, model results indicate that within three generations of the cessation of gene flow, the riparian population would be free of mixed genotypes and moving towards genetic differentiation.

Introduction

Most evolutionary biologists agree that the speciation process requires the cessation of gene flow. While this is generally thought to occur in animal systems through geographic isolation (genetic divergence during allopatry) (e.g., Mayr, 1988; Barraclough & Vogler, 2000), there is inferential evidence, at least, that other prezygotic barriers can lead to sympatric speciation. The process of sympatric speciation in a sexually reproducing animal system first involves adaptation to different local environments or fidelity to different resources within a habitat (reviewed in Bush, 1994). Postzygotic barriers to subsequent introgression might be encountered in the mixing of these genetically divergent types. This represents a costly wastage of gametes (offspring production) and if a mutation were to arise that permitted mate discrimination, gamete incompatibility or some other prezygotic

barrier, it should rapidly spread through the system. Once a prezygotic barrier is in place, the genetic differentiation of local populations into distinct species can more readily occur, because effectively the prezygotic barrier acts much as a geographic barrier would in limiting population mixing.

Riechert (see 1999 review) has examined a desert spider system in which there is local population differentiation in a suite of behavioral traits that adapt individuals to two very different competitive and predatory risk environments. Riparian habitat patches associated with springs and waterways afford spiders living there high prey levels but high risk of predation. The riparian habitat patches are imbedded in a mosaic of more arid habitats offering low prey levels and low predation risk. Riparian habitats favor a non-aggressive phenotype, while the arid habitats favor a competitive aggressive phenotype. Phylogeographic studies are in progress to determine which population

type (non-aggressive v.s. aggressive) is ancestral. Regardless of the outcome, the spider system appears to be a snapshot of potential sympatric speciation in progress (*sensu* Bush, 1994). Gene flow from surrounding low prey, arid habitats (competitive environments) has experimentally been demonstrated to limit behavioral adaptation in spiders occupying low competition but high predation risk riparian habitats (Riechert, 1993a; Maupin & Riechert, 2001). Also, gene flow levels are sufficiently high as to categorize local populations of *A. aperta* in the habitat mosaic studied as panmictic (Riechert, 1993a). Finally, there is no indication that barriers to gene flow have existed between desert riparian and arid habitats in the southwest US: riparian areas have merely been decreasing in extent in recent times (Smiley et al., 1984). In this study, we quantify levels of gene exchange in a riparian population of *A. aperta* with particular reference to the potential operation of prezygotic and postzygotic barriers to gene exchange.

The spider system

The arid-land spider, *A. aperta* (Gertsch) (Araneae: Agelenidae) is an annual species whose life history is adjusted such that it reproduces just prior to the onset of the desert summer rains. Previous work on the species has shown that most populations exhibit behavioral traits that adapt individuals to low levels of prey availability and restricted feeding periods. The spiders are aggressive towards prey and towards each other in defense of sites that offer high prey availabilities and favorable thermal environments. Individuals belonging to this 'arid-land phenotype' are also quick to return to foraging following the presentation of a predatory cue. Spider 'aggressiveness' towards prey, predatory cues and conspecifics reflects the pleiotropic effects of the same genes (Riechert & Hedrick, 1993). Relative aggressiveness is determined by two antagonistic traits 1) the 'tendency to flee' is an autosomal, quantitative trait, while 2) the 'tendency to attack' is inherited on the sex chromosomes (Maynard Smith & Riechert, 1984; Riechert, 1987; Riechert & Maynard Smith, 1989).

An alternative ecotype of *A. aperta* inhabits desert riparian areas that are generally narrow strips of deciduous dry woodland located on either side of a streambed. Behavioral traits that are adaptive to individual spiders occupying this habitat are the reverse of those required of the species in its more typical, highly competitive environment. The 'riparian phenotype'

is non-aggressive towards abundant prey (Riechert, 1991). Riparian spiders also are non-competitive towards conspecifics, as web sites providing high levels of prey and favorable temperatures and humidities are abundant in this habitat (Riechert, 1979, 1981). On the other hand, risk of predation by birds is great and a spider exhibiting the 'riparian phenotype' is slow to return to foraging following the presentation of a predatory cue (Riechert & Hedrick, 1990).

Empirical studies demonstrate that riparian *A. aperta* often fail to exhibit the 'riparian' behavioral phenotype predicted for them (e.g., Hammerstein & Riechert, 1988). A riparian population in southeastern Arizona (AZ riparian) also shows more variability among individuals in the behavioral traits discussed above than observed in arid-land populations of the same species (e.g., Riechert, 1993b; Maupin & Riechert, 2001). Population genetic analyses indicate that gene exchange between the AZ riparian population and surrounding populations in more arid habitats has occurred. Further, drift fence censuses of *A. aperta* movement (this spider does not exhibit aerial ballooning) indicate that gene flow is largely unidirectional from more arid habitats into the more mesic riparian site (Riechert, 1993a). In an enclosure experiment Riechert (1993a) restricted gene flow while exposing treatment spiders to the high food level and high predation pressure environment typical of the riparian habitat. Within one generation of selection in this environment in the absence of gene flow, there was a marked shift in the treatment enclosures towards the non-aggressive riparian phenotype expected for the habitat (Riechert, 1993a). The shift was not observed in the enclosures protected by netting from avian predators. Using a reciprocal transplant experiment, Riechert and Hall (2000) found that riparian spiders do not survive to reproduction in the arid dry woodland habitat. However, the selection differential against dry woodland spiders in the riparian habitat is about 0.5 when compared to the survival of native riparian spiders in this habitat.

In the study reported here, we used marked population censuses, drift fence enclosures, and laboratory breeding experiments to quantify the level of gene flow occurring between neighboring arid-land and AZ riparian *A. aperta*. While we did not find evidence of a significant prezygotic barrier to arid-land and riparian population mixing, we did identify a postzygotic barrier to introgression, which produces significant levels of gamete wastage. Using the results of the empirical study we develop a model to quantitatively explore the

consequences of genetic mixing between riparian and arid-land phenotypes of *A. aperta*.

Methods

The tracking of gene flow from arid-land adapted spiders into a riparian spider population involved a complex series of field and laboratory studies. Table 1 provides a step-wise summary of the procedures used in examining the various factors that might limit the mixing of riparian and arid-land genotypes.

Study areas

The fieldwork was completed on the grounds of the Southwestern Research Station in the Chiricahua Mountains of southeastern Arizona: Cochise County (elevation = 1610 m). *A. aperta* occupies two habitats at the site, desert riparian and evergreen (dry) dry woodland (classification of Lowe, 1964). The riparian habitat is a mesic one that has a closed canopy of walnut (*Juglans major* (Torr.)), Arizona sycamore (*Platanus wrightii* Wats.), box elder (*Acer negundo* L.), cottonwood (*Populus deltoides* Marsh.), and alligator juniper (*Juniperus Deppeana* Steud.). The forest floor consists of patches of rock piles and horsetail rush (*Equisetum hiemale* L.) interspersed in a grass and leaf-litter matrix. It lines north and south sides of a permanent spring-fed stream, Middlefork Creek, extending approximately 50 m to the south of the stream and 70 m to the north. This flat expanse of mesic habitat ends fairly abruptly at the base of an 18 m high, steep slope that levels off into an evergreen dry woodland.

The evergreen (dry) dry woodland is sufficiently removed from the influence of the stream to be classified as an arid environment. It is dominated by widely dispersed live oaks (*Quercus Emoryi* Torr. is the most common species) and the pine, *Pinus leiophylla* var *chihuahuana* Engelm., is also present. The live oaks and pines provide little shade and there is no litter accumulation. The substrate, thus, consists of sparsely distributed grasses on bare ground and gravel.

Prezygotic barriers to gene flow

Habitat isolation: immigration estimates

As *A. aperta* is active in southeastern Arizona between mid-February and October, we completed our fieldwork during this time period. During 1995 and 1996 field seasons we investigated potential habitat isolation

by studying the movement patterns of dry woodland (a total of 314 individuals) and riparian spiders (a total of 1,334 individuals). This included drift fence tracking of the immigration of dry woodland spiders into a 1 ha enclosed area of riparian habitat. All individuals captured within this enclosed riparian area and adjacent area of dry woodland habitat were aged and sexed, and were individually paint-marked with dots of enamel paint applied to the dorsum of the abdomen using a single hair of a paintbrush. We returned each individual to its flagged web site at this time. We inspected the study areas each day for spider molting events and web relocations. Individuals were recaptured and their markings renewed with each molt during the course of the growing season. Each new site was flagged denoting the identity of its occupant.

Enclosure design. The riparian area used in this study was enclosed so that we could monitor the immigration of dry woodland spiders into a local marked population of riparian spiders. This area (Figure 1) was bordered on the north by Middlefork Creek, which provided a natural barrier to immigration. A 0.5 m high drift fence wall enclosed the riparian study area on the other three sides, S, E and W (Figure 1). The frame of the enclosure wall was constructed of PVC pipe (diameter = 2.54 cm) that was mounted on iron rods (diameter = 0.63 cm) pounded in the ground to a depth of 0.25 m. Landscape-grade plastic sheeting attached to the PVC pipe frame formed the enclosure walls. The bottom of each wall was buried in the substrate. A 30 cm wide ledge of aluminum flashing was centered on the PVC pipe forming the top edge of the enclosure walls. The ledge provided a horizontal barrier to dispersal of 15 cm on both inner and outer sides of the enclosure. A non-drying glue (Pest Glue, Seabright Industries) was spread on the underside of the flashing ledges to further guard against unmonitored *A. aperta* dispersal from or into the enclosures. From previous work with sticky barriers (e.g., Singer & Riechert, 1995), we learned that *A. aperta* does not attempt to cross these barriers.

Figure 1 shows the placement of lines of 'live' pitfall traps in the study area that were used to monitor spider movement and to obtain information on distances traveled by immigrants and natives in the riparian habitat. The pitfalls (Figure 2(a)) were buried flush with the substrate surface and were placed at 0.5 m intervals along the walls shown. We checked the pitfall lines for *A. aperta* at the end of morning and evening activity periods which were delimited by

Table 1. Summary of studies completed in tracking arid-land spider gene flow into a riparian population of *Agelenopsis aperta*

Foundation studies	¹ Behaviorally adapted local populations: arid-lands = aggressive; riparian = non-aggressive (Riechert, 93c) ² Selection differential phenotype in foreign habitat: arid-land = 0.5; riparian = 0 (Riechert & Hall, 2000) ³ Immigration = biased: arid-land into riparian (Riechert 1993)	
This study	Tracking gene flow of arid-land phenotypes into riparian population	
I. Prezygotic reproductive barriers:		
Habitat isolation	¹ Spider tracking: population comparison of frequency of moves and distance traveled	² Drift fence/riparian habitat enclosure: rate of immigration of arid-land immigrants into riparian habitat by sex
Behavioral isolation		
Incorporation into population deme	¹ Spider tracking within enclosed riparian habitat: females hold web sites as sexually mature adults	² Spider tracking within riparian habitat enclosure: males as adults visit sexually mature females
Mating success	¹ Females collected from web sites in riparian habitat enclosure and scored for aggressiveness before released into mating pool ³ Mating success trials in laboratory: reciprocal tests for pure and mixed behavioral phenotype pairings	² Female web site checks for matings in riparian habitat enclosure
Mechanical isolation	¹ Laboratory breeding trials involving pure and mixed phenotype matings: egg sac viability estimates	² Laboratory breeding trials F1 generation hybrid matings and backcross matings of hybrids to parental lines: egg sac viability estimates
II. Postzygotic reproductive barriers:		
Hybrid inviability	¹ Laboratory breeding trials involving pure and mixed phenotype matings: egg sac viability and survival to sexual maturity estimates	² Laboratory breeding trials F1 generation hybrid matings and backcross matings of hybrids to parental lines: egg sac viability and survival to sexual maturity estimates
Hybrid sterility	¹ F2 generation hybrid and backcrosses of hybrids to riparian and dry woodland parental line breeding experiments: egg sac viability and offspring survival estimates	

Table 1. (continued)

Genetic barriers to introgression	¹ Females collected from web sites in riparian habitat enclosure and scored for aggressiveness before released into mating pool ³ F1 Behavioral assays completed on F1 generation dry woodland spiders and reciprocal crosses between this population and riparian spiders: fit to genetic model of <i>A. aperta</i> aggressiveness	² Female web site checks for matings in riparian habitat enclosure relative to behavioral phenotype ⁴ Behavioral assays completed on F2 generation hybrids of riparian X dry woodland parental lines and backcrosses to these parental types: fit to genetic model of spider aggressiveness (i.e., production of extreme phenotypes)
III. Gene flow levels and effects	¹ Simulation of gamete wastage under different levels of immigration	

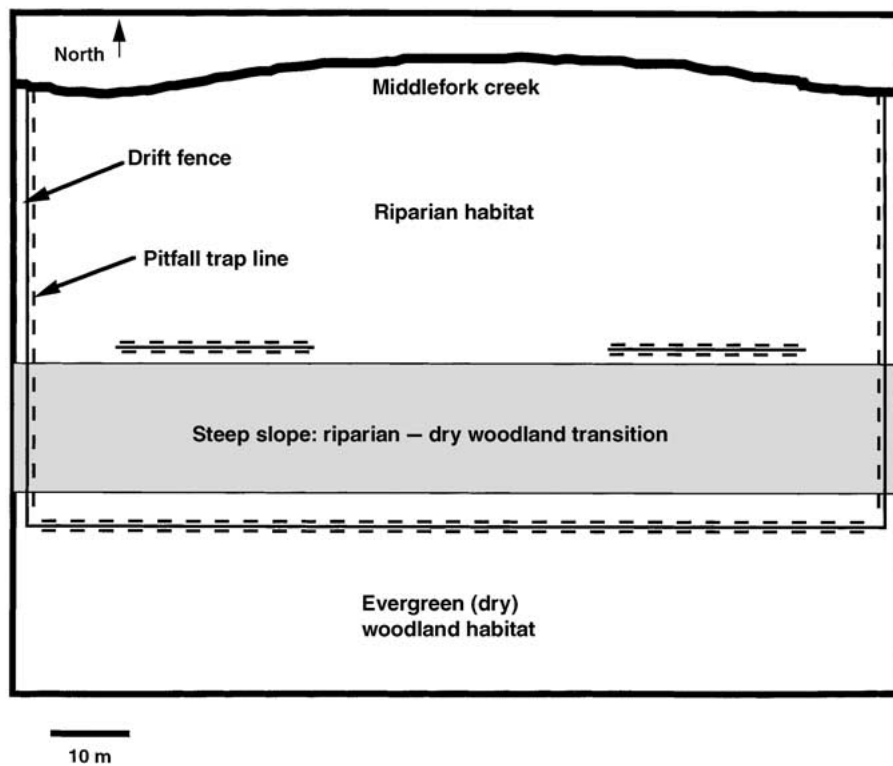


Figure 1. Study area used in obtaining estimates of gene flow between riparian habitat natives and dry woodland immigrants. The 1 ha area is bounded on north by creek and on other three sides by enclosure walls of a drift fence (—) to which a sticky barrier (Pest Glue[®], Seabright Industries) was regularly applied at the top. Dashed lines show location of pitfall trap lines along the drift fences: pitfalls were placed at 0.5 m intervals on only the inside edge of east and west enclosure walls, but on both sides of internal drift fences and south enclosure wall that separates riparian from dry woodland habitat.

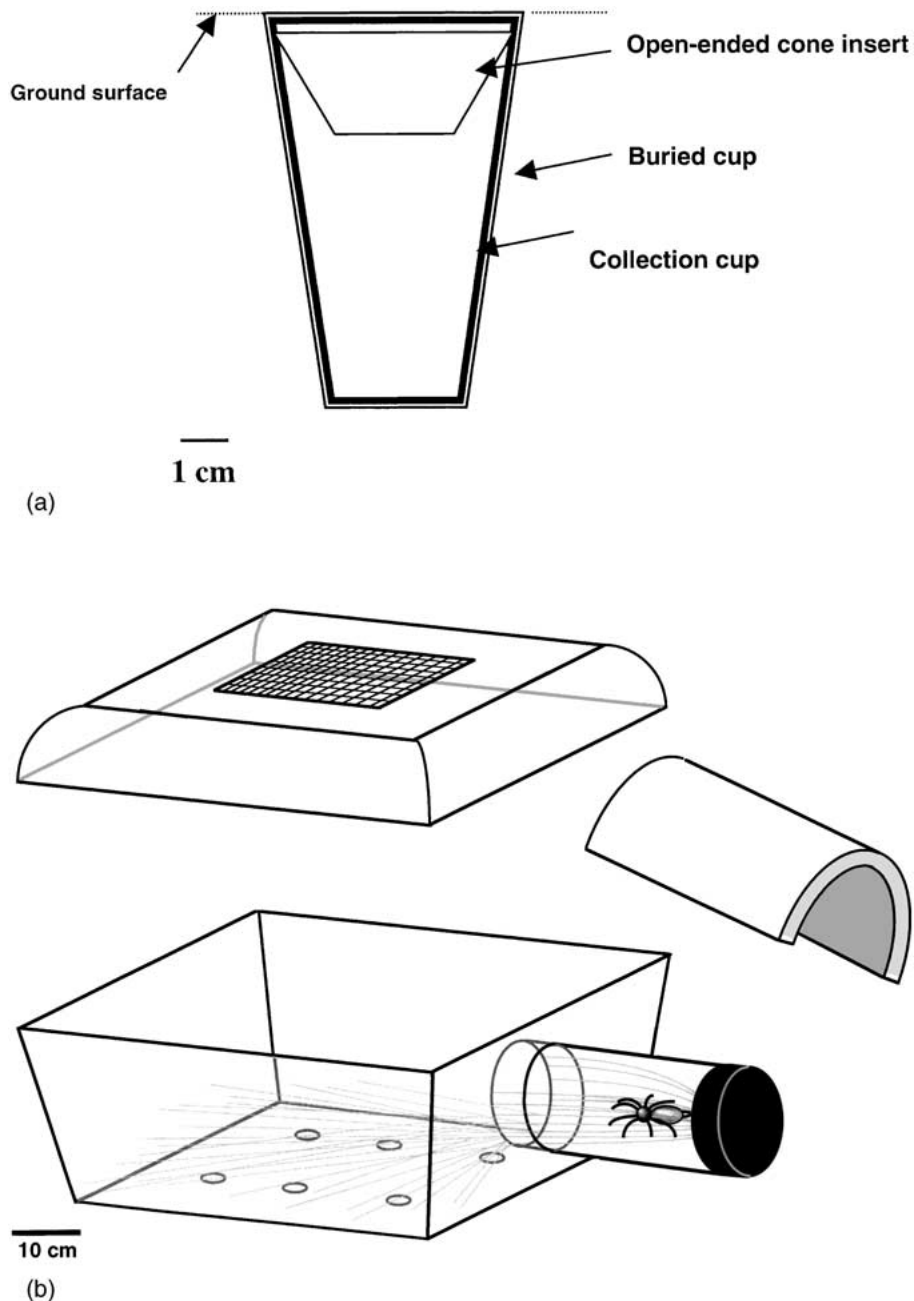


Figure 2. Designs of tools used in this study. (a) Pitfall trap consists of two 0.95-l plastic containers and a plastic cone insert. The plastic container designated as the collector is inserted in a container buried flush with the ground surface at the edge of a drift fence. The plastic cone with a 3 cm wide hole is positioned at the top of the collector cup to prevent escape through climbing. (b) Fabricated web site consists of a rectangular plastic box with domed screen lid and associated tube funnel. Floor of web chamber is perforated to prevent accumulation of water during rains. Lid of box is removed after the spider has built a funnel in the tube, a web in the box, and the box has been situated back at the field site from which the spider had been collected. At the site, a layer of fiberglass insulation laid over tube protects web funnel from temperature extremes. Insulation is lifted for inspection of the web-funnel during censusing.

temperatures falling between 18 and 31°C (Riechert & Tracy, 1975). We used a 50 m tape to measure the linear distances moved between web sites, between pitfall trap locations, and between pitfall locations and web sites for each move noted for a marked spider.

Most of the pitfall trap lines (i.e., east and west walls of the enclosure and two 30 m lines at the base of the hill that demarcated the transition between riparian and dry woodland habitat) were used only to intercept moving individuals for the purpose of obtaining distances traveled. We weighed spiders collected in these traps, checked their identities (paint-mark), and recorded their capture location before we released them back into the enclosure. We did not release captured individuals in the immediate vicinity of the drift fence and pitfall trap as this would lead to their immediate recapture. Rather we released them at a standard distance of 3 m from the trap location towards the enclosure center.

The pitfall lines located along the southern wall of the enclosure (Figure 1) served an additional function to the monitoring of spider movement and distances traveled. This drift fence permitted the interception and monitoring of all migration between dry woodland and riparian habitats. We kept a record of all dry woodland spiders immigrating into the riparian habitat enclosure and followed their success in the habitat through the remainder of the field season. At first capture of an unmarked individual in a pitfall on the south side of this drift fence, we recorded the site of capture and date and then weighed and individually paint-marked it as an immigrating dry woodland spider. We permitted riparian individuals captured in pitfalls to leave the enclosure and return to the enclosure along the south boundary as well. In this case, we recorded the pitfall location, direction of movement, and date before release at 3 m on the opposite side of the drift fence from that of capture.

Behavioral isolation: tests for mating success

In addition to the daily census of web sites and day and evening censuses of pitfall trap lines, we completed checks of female web-sites at 30 min intervals during the mating period, which started in July and continued into October. This intensive censusing was necessary to establish the relationship between rate of dry woodland spider immigration and the rate of mixed phenotype matings in the riparian habitat.

We used a monitoring protocol already developed to investigate the mating system of *A. aperta* in a desert grassland habitat area in New Mexico (Singer

& Riechert, 1995). As individuals possessing web sites within the enclosure approached maturation, we collected them and brought them into the laboratory to establish them in boxes with attached funnels that permitted the viewing of courtship and mating (Figure 2(b)). While maintained in the lab, the spiders were fed *ad libitum* food every 3 days. At the time of capture, we recorded the compass orientation and vertical angle of the web funnel and height of the web sheet above the substrate. We also marked the web site with a permanent flag denoting the number assigned to its resident and its unique color code. This information was used later in re-establishing the individual at its web site.

Because the riparian population is continually subject to gene flow from surrounding arid habitats, knowledge of the behavioral phenotype of each spider collected from riparian habitat is important to our understanding of its mating success. Thus, we completed two separate behavioral tests on each individual while it was maintained in the laboratory: response to predatory cue/fear and agonistic/contest behavior tests. The tests described below permitted us to establish the position of a particular individual on a continuum from non-aggressive to highly aggressive. A spider was available for behavioral trials when two conditions were met: (1) it had established a web in its home box (Figure 2(b)), and (2) 2 days had passed since an *ad libitum* feeding.

Predatory cues test. This test (first described in Riechert & Hedrick, 1990), assessed spider response to the large amplitude deflection of the web that would be caused by the approach of an avian predator. The test was completed on individuals that were positioned at their funnel entrances facing their web traps. In preparation for a trial, we removed the lid of the container housing the spider. A minimum interval of 5 min was used as a potential settling time following this disruption. At initiation of the trial, we directed four puffs of air from the bulb of a camera-cleaning brush towards the spider. The bulb was held at a 45° angle to the web at a height of 8 cm above the sheet and was positioned at a distance of 6 cm in front of the funnel entrance. These puffs mimicked the cues *A. aperta* might receive from an incoming avian predator (Riechert & Hedrick, 1990). We scored the spider as to how long a period of time has elapsed between its retreat into the web-funnel in response to the cue and its return to a foraging mode at the funnel entrance. Failure to retreat within four puffs was scored as a

0-s latency to return to foraging. No retreat or a short latency to return to foraging is associated with exhibition of the more aggressive arid-land phenotype while a long latency to return to foraging is characteristic of the non-aggressive riparian phenotype (Riechert & Hedrick, 1990).

Agonistic behavior trials. From previous studies (Riechert, 1979, 1993a), we learned that *A. aperta* readily compete for webs built by other individuals. In this test of spider aggressiveness in contests, we simultaneously introduced individuals matched for weight and sex to a web built by a third individual who had been removed earlier. We placed the open plastic box (15 cm in diameter and 6.5 cm in height) housing the web in a clear, rectangular box (16 cm × 30 cm × 10 cm), which permitted the withdrawal of the losing spider from the web without escape.

At trial initiation, we introduced both individuals into open-ended syringes. We then introduced one of the two individuals to the empty web. When it ceased movement after introduction, we introduced the other member of the pair at the opposite end of the web sheet. (Individuals retreating from the web at this introduction stage were reintroduced.) We observed the contest for 10 min following the first interaction between the two individuals. If the contest outcome was not completed within this initial time period, we placed the lid on the outer box and checked the contest at 15 min intervals for determination of contest outcome. We recorded the date, sex, color marking, and weight of each contestant, the order in which they were introduced, and which individual won the contest. The winner was designated as the individual in control of the funnel after the other contestant had withdrawn from the web into the outer box. If both individuals had withdrawn from the web, the contest was scored as a draw, and if neither individual withdrew but rather shared the web for 24 h, a no-contest designation was assigned to the interaction. This protocol was first described in Riechert (1993a).

Measuring mating success in the field. When a spider completed its final molt to maturity, we reintroduced it to its former web site in the field. We released males only in the tubes housing their funnels as males abandon webs at this stage. We individually situated each tube housing a male and the home box with attached tube housing a female (Figure 2(b)) in the substrate to achieve the funnel orientation and angle

and web sheet height above the substrate noted for the spider at capture. We covered the exposed upper surface of the web funnel with foil-covered, fiberglass insulation (Figure 2(b)). The insulation provided a dark funnel environment and protected the spider from temperature extremes.

The screened lid of the plastic box housing the female's web was removed 24 h after the spider had been transplanted from the lab to its field site. At this time, we included this spider in the continuous census we made of each active web within the enclosure. To determine whether a mating was occurring in the funnel, we removed the layer of insulation from the plastic tube at least once every 30 min of the activity period of *A. aperta*, which is restricted to air temperatures of 18–31°C (Riechert & Tracy, 1975). We noted all instances of sexual cannibalism found during the censusing. We recorded the identity of the perpetrator and whether the cannibalism occurred prior to mating (during courtship) or after mating.

Sexually mature female *A. aperta* release the pheromone 8-methyl-2 nonanone that both attracts males to their webs and elicits courtship (Papke et al., 2001). Upon making contact with a mature female's web, the male exhibits various vibratory courtship displays (Riechert & Singer, 1995; Singer et al., 2000). The visited females may respond by attacking the male or permitting him to continue courtship. Mating usually takes place within 2 h of the male's arrival (mean latency period to court = 3.5 min; mean courtship duration = 83.4 min Singer et al., 2000). Copulation may continue intermittently for up to 2 days (mean duration = 863.7 min) (Singer & Riechert, 1995). Given the long courtship period and extremely long period of mating, it is unlikely that our census interval missed any successful matings.

Laboratory measurements of mating success. Over a 4-year period, starting in 1995, approximately 400 immatures of each of the two local populations, riparian and dry woodland, were collected and raised to sexual maturity in the laboratory. These spiders were collected from respective dry woodland and riparian habitats outside of our field site. All feral individuals were maintained in individual plastic containers (16 cm × 30 cm × 10 cm) and fed *ad libitum* food two times per week during this period. We waited until 2 weeks post molt (age of sexual maturity Riechert & Singer, 1995) to offer males to females in the following categories: dry woodland X dry woodland, riparian X riparian, riparian female X dry woodland male,

and dry woodland female X riparian male. Individuals were assigned to category by random draw and no individual was used in more than one trial. All mating trials were completed in the home box of the female spider. In a subset of these matings we recorded the outcome of the interaction (mate v.s. no mate).

Mechanical isolation: tests for zygote viability

Egg sac viability determinations were made on the 1st egg cases produced by females mated in the laboratory. Spiderling emergence was used as the criterion for viability. We included only the 1st sac produced because females in nature rarely produce more than one clutch of eggs (Riechert & Tracy, 1975). We also inspected each egg case after emergence for partial hatches and weighed *en masse* 20 spiderlings from each sibling class to obtain an estimate of mass/individual.

Postzygotic barriers to gene flow

Hybrid inviability and sterility: laboratory studies of survival and reproduction

We subsequently reared the 20 individuals weighed in the test for mechanical isolating mechanisms to maturity. Briefly, the rearing protocol involved isolated rearing in individual plastic containers that increased in size with age and thus size of the spiders. We housed the spiders in a controlled environmental chamber at a light and temperature cycle of 12 h light (27°C)\12 h dark (21°C). We fed them *ad libitum* prey levels at 3-day intervals. Young spiderlings received termites, 2-day-old crickets and drosophila; later instars received european crickets, mealworms and moths. We obtained survival estimates for each sib group.

Random matings were performed on these F1 lab reared individuals to obtain similar viability and survival estimates for backcrosses of the hybrids to the parental populations and F2 hybrid generation hybrids.

Genetic barriers to introgression

Laboratory breeding studies of spider aggressiveness. It was important to determine whether the dry woodland local population conforms to the arid-land phenotype previously established for a desert grassland population from south-central New Mexico (NM desert grassland). NM desert grassland *A. aperta* exhibit higher levels of escalation and greater persistence in contests than do AZ riparian *A. aperta* (Maynard Smith & Riechert, 1984; Riechert & Maynard Smith,

1988). Spiders exhibiting the arid-land phenotype are, thus, predicted to win contests over spiders exhibiting a riparian phenotype. In the predatory cues trials, NM desert grassland spiders exhibited faster latencies to return to foraging and they demanded larger territories than did AZ riparian spiders (Riechert, 1979). Crosses made between desert grassland and riparian *A. aperta* produced hybrids that showed levels of aggression that were higher than either parental population (Maynard Smith & Riechert, 1984). The higher aggressiveness of hybrids between desert grassland and riparian *A. aperta* was attributed to directional dominance in the inheritance of 'tendency to flee' and 'tendency to attack' in arid-land versus riparian phenotypes. The genetic model has been substantiated through further breeding experiments involving the two geographically separated populations. Analysis of the aggressiveness exhibited by F2 generation hybrids and backcrosses to parental types indicate that tendency to attack is inherited on the sex chromosomes, while tendency to flee is a quantitative trait inherited on the autosomes (Riechert & Maynard Smith, 1989). Because there is considerable sorting out of phenotypes in the F2 generation hybrids and back crosses, Riechert and Maynard Smith (1989) concluded that the number of genes involved in determination of aggression is limited and the mendelian model applied to the system appropriate.

No genetic studies have been completed on the local population inhabiting the AZ dry woodland habitat. They are necessary to determine whether local populations adapted to different environments show the same directional dominance effects noted for the 'wide cross' between geographically separated populations. To date, Riechert (1973a) has established that: (1) spiders in the dry woodland habitat are subjected to similar selection pressures as are NM desert grassland *A. aperta*, and that (2) field collected spiders from the AZ dry woodland show similar levels of escalation in contests, predatory cue test scores, and attack rates towards prey of high versus low profitability to those exhibited by NM desert grassland spiders.

We completed the agonistic behavior trials described in the field study section on lab reared F1 generation spiders to test the genetic model on AZ local population crosses. These were between-genetic class contests: individuals were bouts against individuals of the same sex and body mass, but of unlike genetic class. In all, five sets of agonistic trials were completed on F1 generation spiders: riparian versus dry woodland, dry woodland X riparian hybrid versus

riparian, riparian X dry woodland hybrid versus riparian, dry woodland X riparian hybrid versus dry woodland, and riparian X dry woodland hybrid versus dry woodland. Note that by convention, the female parent is the 1st individual listed in a cross. The spiders used in the agonistic behavior trials were those reared in the investigation of hybrid viability relative to that of the parental lines (see rearing protocol under *Hybrid inviability and sterility: laboratory studies of survival and reproduction*).

Additional contests were staged between the offspring of individuals that exhibited sexual cannibalism during mating in the laboratory and between control offspring (no sexual cannibalism exhibited in their production). These trials were completed to learn whether sexual cannibalism has a genetic influence.

Model of migration of arid-adapted alleles into a riparian population

We developed a model to investigate the influx of dry woodland-adapted alleles into a riparian population. Punnett squares based on the directional dominance genetic model described earlier (Maynard Smith & Riechert, 1984; Riechert & Maynard Smith, 1989) were used to create transition matrices for the production of female and male offspring genotypes (see Table 7). The assumption was made that a single male sires all of the offspring produced by a female. This assumption is based on the first male sperm precedence noted for this spider and the fact that females cease pheromone release after mating (Riechert & Singer, 1995).

The riparian population was modeled at a carrying capacity of 1000 individuals and initially seeded with 500 males and 500 females of the riparian genotype. Each generation, dry woodland males and females immigrated into the riparian habitat and a proportion of them survived to become members of the breeding population. The numbers of males and females of a given genotype i in subsequent generations were calculated from current generations by the equations:

$$Male_{i,(t+1)} = \sum_{m=0}^5 \sum_{f=0}^8 (P_{m,f} * N * B * Jm_i * Sm_i)$$

$$Female_{i,(t+1)} = \sum_{m=0}^5 \sum_{f=0}^8 (P_{m,f} * N * B * Jf_i * Sf_i)$$

where,

$P_{m,f}$ = the probability of a mating between a male of genotype 'm' with a female of genotype 'f', that

is the product of the proportions of individuals of each genotype, including migrants (see Table 7 for the specific genotype indices),

N_t = population size at time t ,

B = average brood size,

Jm_i = proportion of a brood that are males of genotype i produced from a male/female mating of genotypes m/f ,

Jf_i = proportion of a brood that are females of genotype i produced from a male/female mating of genotypes m/f ,

Sm_i = proportion of male juveniles of genotype i that survive to reproduce,

Sf_i = proportion of female juveniles of genotype i that survive to reproduce.

These equations were solved recursively using C⁺⁺. The model was run for 25 generations, though equilibrium was reached in fewer than 10 generations. We assigned probabilities of survival through reproduction of 0.22 for the riparian phenotype, 0.10 for the arid phenotype, 0.05 for the F1 hybrid (arid X riparian) phenotype, and 0.00 for both the chicken and cannibal phenotypes. The two latter phenotypes, 'chicken' and 'cannibals' are extremes first identified in this study (reported under the results of the breeding censuses completed in the enclosed riparian study area). Chickens are individuals that are so non-aggressive that they retreat from all potential mates, while cannibals are individuals that are so aggressive that they attack every potential mate. These phenotypes arise primarily in F2 generation hybrids and backcrosses to parental lines (see Table 7). The parameter values for riparian, arid and F1 hybrid phenotypes were extrapolated from selection coefficients available from a reciprocal habitat transplant experiment (1st three Riechert & Hall, 2000) and for chickens and cannibals from the empirical findings of this study. The model population was held at a carrying capacity of 1000 individuals.

Results

Prezygotic barriers

Habitat isolation: immigration estimates

At sexual maturity male *A. aperta* abandon their webs and initiate the search for females. This brings dry woodland males into the riparian habitat. Juvenile and mature female *A. aperta* of arid-land phenotype

may also migrate into riparian habitat as a result of shifting web sites. The numbers of dry woodland spiders that immigrated into the enclosed riparian study area varied significantly between the typical dry year of censusing (1995) and the exceptionally wet year (1996) (chi-square test: $X^2 = 15.1$, $df = 1$, $p < 0.0001$). Although male immigrants outnumbered female and juvenile immigrants by 1.6/1.0, the fact that 3.3 times as many females and juveniles moved into the riparian area in the dry year compared to the wet year was largely responsible for the significant chi-square test result. An ANOVA completed on the probability of a juvenile or adult female leaving its web site identified significant year (F ratio = 6.4, $df = 2$, $p < 0.01$) and population (F ratio = 27.6, $df = 2$, $p < 0.00001$) effects, and an insignificant population by year interaction (F ratio = 2.8, $df = 2$, $p = 0.09$). The dry woodland web owners moved web sites more frequently in the typical dry year of censusing (mean proportion moving/day = 0.23 ± 0.02) than they did in the wet year (0.14 ± 0.01). The riparian spiders moved sites less frequently than did dry woodland spiders and did not show variation in the frequency of moves made between the typical dry year (mean proportion = 0.08 ± 0.01) and the exceptionally wet (0.06 ± 0.02) year.

Our data on the distances moved by spiders is limited to the linear distance between sightings or capture points. Thus, the results presented here are low estimates of real distance traveled in a move. The greater distances sexually mature males travel in search of matings than females and juveniles in search of web sites produced a significant ANOVA whole model test result for distance traveled as a function of age and sex class, population and year (F ratio_{11,548} = 17.2, $p < 0.0001$). Males traveled an average of 22.6 ± 1.6 m per move while females traveled 7.4 ± 1.6 and juveniles 6.2 ± 1.5 m. The longest distance traversed in this study was an 85.0 m move by an adult male from dry woodland habitat. While there was a significant sex effect on distance moved (F ratio = 59.7, $p < 0.0001$), with one exception other effects and interactions among them were insignificant at a cutoff alpha of 0.05. The exception was a significant interaction of sex, population and year (F ratio = 5.0, $df = 2$, $p < 0.007$). This result reflected the fact that dry woodland males traveled longer distances in the more typical dry year of censusing (mean distance/move = 30.7 ± 4.6 m), than they did in the exceptionally wet year (mean = 15.1 ± 1.3 m).

Behavioral isolation: tests for mating success

Field estimates. The relationship between establishment in the potential breeding population (deme), and mating success for dry woodland spiders in the riparian habitat is shown in Figure 3 for the 2 years of field monitoring. We assigned membership to the riparian population deme to an immigrant dry woodland male if it was observed visiting sexually mature females in the riparian habitat during the course of censusing. An immigrant dry woodland female was assumed to be part of the riparian population deme if it held a web site in the enclosed riparian area as a sexually mature individual. While no matings involving two dry woodland partners took place in the riparian habitat during the 2 years of monitoring, we did find that between 4 and 10% of the matings there involved a dry woodland immigrant and a riparian mate. There was a higher level of mixed local population matings in the typical dry year than in the exceptionally wet year (Figure 3(b)). This corresponds to the higher level of immigration already noted of dry woodland *A. aperta* into the riparian habitat in the dry year. It further reflects the relationship between immigration and incorporation into the population deme, as there was a greater representation of dry woodland immigrants in the population deme in the dry year than in the wet one (Figure 3(a)). There were no significant differences in the ratios of arid-land immigrant mating success/deme membership for either sex or year (p -values ranged from <0.60 to 0.99 McNemar tests for difference in proportion).

The field results indicate that matings by immigrant dry woodland spiders with native riparian spiders is proportional to the visitation rate which, in turn, is proportional to the immigration rate of dry woodland spiders into riparian habitat.

Mating trials in the laboratory. The results of laboratory mating trials provide both corroborative evidence for the field monitoring results and establish the fact that there is no behavioral barrier to gene flow in place. The total of 309 mating trials completed in the laboratory were partitioned among the four categories: within riparian population, within dry woodland population and reciprocal dry woodland female X riparian male and riparian female X dry woodland male trials (Table 2). A chi-square test completed on the frequency of successful versus non-successful matings among the different classes of individuals supports the field result of no discrimination of phenotype by *A. aperta* ($X^2 = 5.2$, $df = 3$, $p > 0.10$).

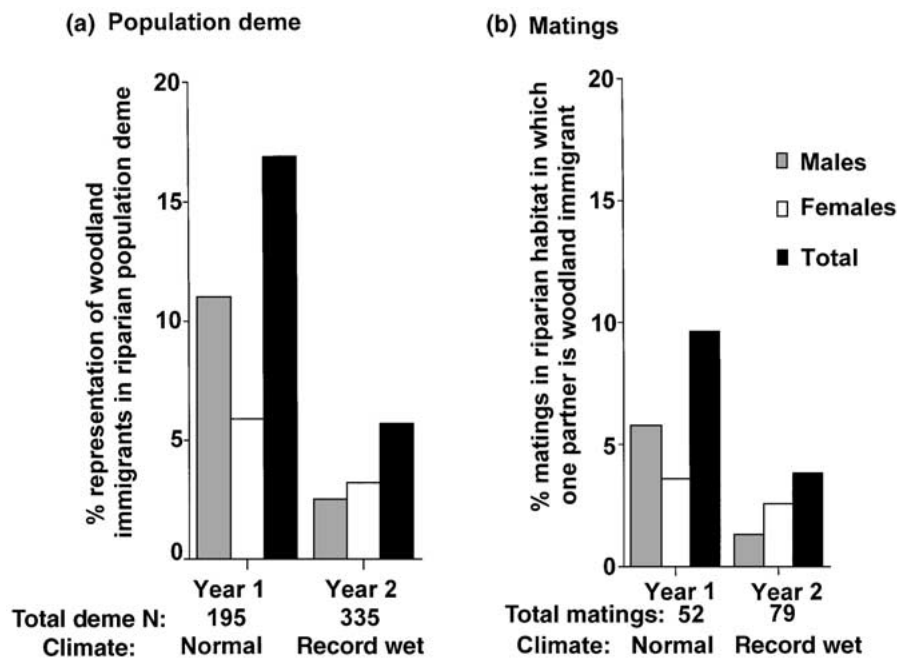


Figure 3. Dry-dry woodland immigrant influence on riparian spider population (a) proportion of population deme, (b) proportion of matings. Year and sex comparisons.

Table 2. Laboratory test for a behavioral prezygotic barrier to population mixing: mating trial results for different local population combinations of sexually mature *Agelenopsis aperta*

Female class	Male class	Absolute (relative) frequency	
		Mate (%)	No Mate (%)
Riparian	Riparian	77 (61)	50 (39)
Dry woodland	Dry woodland	41 (73)	15 (27)
Riparian	Dry woodland	38 (66)	20 (34)
Dry woodland	Riparian	73 (74)	25 (26)

Mechanical isolation: tests for zygote viability

All mated females from the laboratory crosses discussed above and from subsequent F2 hybrid and hybrid backcrosses to the parental populations produced at least one egg case (clutch). The majority of the clutches were viable regardless of the class of parentage (Table 3). The frequency of viable versus non-viable 1st egg sac production did not differ significantly among the breeding classes ($X^2 = 1.2$, $df = 5$, $p > 0.50$). Note that partial viability within a clutch was rare. We inspected the empty egg cases following spiderling emergence for unhatched eggs. Only five of the 418 cases from which spiderlings emerged

contained inviable eggs in them. Clearly, there are no mechanical barriers to population mixing.

Postzygotic reproductive barriers

Hybrid inviability

We completed two estimates of subsequent survival of pure versus mixed population lines, as there is no evidence for zygote mortality. Spiderling mass at emergence can be used as an estimate of expected spiderling success, because body mass is a major determinant of prey capture success in young spiderlings (S.E. Riechert, personal observations). A one way ANOVA was used to test for differences in spiderling mass among the genetic classes. The whole model test result was insignificant (F ratio_{5,277} = 1.7, $p = 0.14$) (Table 3). We also examined the survivorship of different genetic classes in the laboratory environment as represented by the percentage of the 20 individuals reared from each clutch that survived to sexual maturity (Table 3). An ANOVA completed on these data did not detect a significant genetic class relationship in survivorship to maturity (F ratio_{5,534} = 1.8, $p = 0.11$). Offspring of mixed population parentage survived in the laboratory environment equally well as offspring from the respective riparian and arid populations.

Table 3. Laboratory tests for a mechanical prezygotic barrier and hybrid inviability postzygotic barriers to population mixing

Class	N	Potential barriers to population mixing		
		Prezygotic mechanical percentage of 1st egg sacs viable	Postzygotic measures of hybrid inviability	
			Spiderling mass at emergence mean \pm SE	Percentage survival to sexual maturity mean \pm SE
Riparian X riparian	82	87.8	0.56 \pm 0.02 mg ^a	39.4 \pm 2.5 ^a
Dry woodland X dry woodland	136	90.4	0.57 \pm 0.04 mg	39.5 \pm 1.9 ^b
F1 hybrid: riparian X dry woodland	73	91.8	0.64 \pm 0.04 mg	47.3 \pm 3.0 ^{ab}
F1 hybrid: dry woodland X riparian	57	93.0	0.67 \pm 0.03 mg	42.4 \pm 3.5
F2 generation hybrids	76	85.5	0.67 \pm 0.03 mg ^a	42.6 \pm 2.5
Backcrosses to parental populations	44	86.4	0.63 \pm 0.02 mg	48.2 \pm 2.5 ^b

Letters indicate pairs of classes that are significantly different at an alpha of 0.05 (Student's t comparison).

We successfully produced viable F2 generation hybrids and backcrosses of hybrids to dry woodland and riparian parental lines (Table 3). This indicates that there is no hybrid sterility, another potential postzygotic isolating mechanism.

Genetic barrier to introgression

Field censuses of matings. We did obtain evidence of a potential genetic barrier to introgression in the results of the censuses of matings summarized in Figure 3. First, we observed that sexual cannibalism (the killing of a potential mate often followed by consumption) occurred in a variety of contexts involving male and female riparian spiders (Table 4). This is particularly interesting when one compares the incidence of sexual cannibalism in the AZ riparian situation to the total absence of the behavior in field censuses of mating in the NM desert grassland *A. aperta* system reported in Singer and Riechert (1995). This latter population occupied a large, continuous expanse of arid habitat and experienced no potential gene flow from riparian habitats. The probability of a successful mating was also very high in the NM desert grassland habitat (76.0%) compared to the AZ riparian habitat (35.6%) (Table 4). We applied a chi-square test to compare the frequencies of female visits by males that resulted in mating in the two populations. The differences between the AZ riparian population and NM desert grassland population in the frequency of male visits that led to successful mating was highly significant ($X^2 = 42.7$, $df = 1$, $p < 0.0001$).

The low mating success observed in the AZ riparian habitat led to closer inspection of the mating

records. As many as five different males were observed to visit a single female during the course of the study. (The number of male visits may be underestimated as our census interval of 30 min would miss males that were quickly repulsed.) We found that an average of 5.2% of the females that were visited by multiple males in the enclosed area attacked all males that visited them. Another 22.0% of the females retreated from the web site every time a male visited (Table 5). We identified (by their color codes) the females that rejected all males. All were native to the riparian habitat in the sense that they had emerged from egg cases within this habitat. Because *A. aperta* is an annual species, attacking all males or running from all potential matings during our census period is equal to 0 fitness. We assigned the highly aggressive class the descriptor 'cannibal', and the excessively non-aggressive class 'chicken'.

We examined the behavior of the females identified above in the agonistic and predatory cues tests completed on all individuals prior to their return to their web sites in the riparian study area as sexually mature individuals (see protocol under the Methods section *Behavioral isolation: tests for mating success*). The behavior the respective extreme classes of females exhibited towards males was highly correlated with the levels of aggression the females exhibited in other contexts (Table 4). While all females designated as cannibal from their behavior toward potential mates won their contests over webs in the agonistic trials, only 6% of the females identified as chicken won their contests. We compared by ANOVA the predatory cues test latencies to return to foraging for the following classes of females: cannibals, chickens, females

Table 4. Comparison of mating success and field rates of sexual cannibalism occurring during courtship between two populations of *A. aperta*: AZ riparian population experiences gene flow from arid-land habitats, while NM arid-land population experiences no gene flow

Population	Frequency of cannibalism						
	General mating statistics			Incidence of sexual cannibalism			
	Visits	Matings	Successful	Females		Males	
	<i>N</i>	<i>N</i> (%)	matings mating	After courtship	During mating	After courtship	During
AZ riparian	421	150	35.6	27	17	3	13 ^a
^b NM arid-land	75	57	76.0	0	0	0	0

^aIn seven of these cases, males killed females without feeding on them.

^bData from field breeding censuses reported in Singer and Riechert (1995).

Data summary for two breeding seasons in each case.

Table 5. Examination of extreme phenotypes exhibited by *A. aperta* females from a riparian population experiencing gene flow from arid-land habitats

	Cannibals attacked all potential mates	Chickens retreated from all potential matings
Proportion (<i>N</i>)		

We consequentially distinguished F2 generation offspring as having a parent that cannibalized a mate versus having no parent that cannibalized a mate (designated as controls). The total of 107 agonistic trials were completed between offspring fostered by parents that exhibited sexual cannibalism and opponents chosen from the control group (offspring of matings in which no sexual cannibalism occurred). The offspring of cannibals won 80.4% of these contests, a highly significant proportion ($X^2 = 39.5$, $df = 1$, $p < 0.0001$). Further, during the course of the latter contest trials, the losing spider was cannibalized with a frequency of 18.7%. The incidence of cannibalism in the general pool of contests completed on various genetic classes of riparian and dry woodland spiders was only 4.7% ($N = 272$). The higher level of cannibalism noted for contests involving the offspring of a parent that exhibited sexual cannibalism was significant ($X^2 = 16.4$, $df = 1$, $p < 0.0001$).

Genetic explanation of extreme behavioral phenotypes

Validation of genetic model of aggressiveness for local AZ population crosses. The model developed by Maynard Smith and Riechert (1984) to explain spider levels of aggressiveness was based on the behavior recorded for an AZ riparian ecotype, an NM arid-land ecotype, and reciprocal crosses between them. We tested the local population cross AZ riparian X AZ dry woodland for the predicted directional dominance effect that was based on the contest results for the wide cross: AZ X NM. Agonistic trial tests were staged in the laboratory between individuals produced by various F1 genetic crosses to test the following predicted dominance relationship in the probability of winning contests over webs:

Females:

F1 hybrids (rip X arid-land/arid-land X rip) >
F1 arid-land > F1 riparian

Males:

F1 hybrids (arid-land X rip) > arid-land >
F1 riparian and F1 hybrid (rip X arid-land),

where in a cross, the first population designates the origin of the female parent.

These dominance relationships were predicted from the results of a genetic model Maynard Smith and Riechert developed to explain the aggressiveness of individuals produced in crossing NM desert

grassland spiders (arid-land phenotype) with AZ riparian spiders (riparian phenotype) (Maynard Smith & Riechert, 1984; Riechert & Maynard Smith, 1989). Agonistic trial data completed in this study on female F1 generation spiders fit the dominance predictions made for them (Table 6). Males met the dominance predictions for their winning of contests over webs as well with the exception of the fact that male dry woodland X riparian hybrids did not have a significant advantage over dry woodland males (Table 6). The frequency distribution of phenotypes and proposed genotypes associated with different crosses are presented in Table 7 to aid the reader in understanding the directionality of these results. The genetic model produced to explain the behavior of two different ecotypes and their crosses fits this local population system equally well as it did the NM desert grassland and AZ riparian comparison (Maynard Smith & Riechert, 1984). The NM grassland population is 300k distant from the AZ riparian population.

Accommodating extreme phenotypes. A brief description of the genetic model originally developed is necessary to understanding the modifications we have made to include two additional phenotypes, chicken and cannibal. The basic model of spider aggressiveness is based on the antagonistic interaction of two gene complexes. The prominent component governs some scalar described as 'tendency to attack'/aggression. Aggression is inherited on the sex chromosomes (Riechert & Maynard Smith, 1989) and while females have two homologues of a pair of sex chromosomes, males have only one. This means that a male *A. aperta* inherits its tendency to attack/aggression from its female parent. The scalar 'tendency to flee'/fear is an autosomal quantitative trait that appears to modulate level of aggression. The model developed in Maynard Smith and Riechert (1984) further predicts the existence of directional dominance in the traits between arid and riparian populations, where high aggression (A) is dominant over low aggression (a), but low fear is dominant (B) over high fear (b). Thus the genotype of a dry woodland spider (arid-land phenotype) would be AA (high aggression) bb (high fear), that of a riparian spider (riparian phenotype) would be aa (low aggression) BB (low fear), and that of an F1 hybrid (hybrid phenotype) would be Aa (high aggression) Bb (low fear).

In extending the genetic model predictions to F2 generation hybrids and backcrosses, we find that females exhibit nine genotypes and males six genotypes.

Table 6. Probability of winning contests by genetic class and sex for F1 generation lab-reared *A. aperta* belonging to different genetic classes

Contest class (Female X male parent)	Female versus female		Male versus male	
F1 hybrid: rip X Wd versus F1: rip X rip	0.74 (17)	$X^2 = 5.3$ $df = 1$ $p < 0.025$	0.52 (10)	$X^2 = 1.2$ $df = 1$ NS
F1 hybrid: rip X Wd versus F1: Wd X Wd	0.70 (19)	$X^2 = 4.5$ $df = 1$ $p < 0.05$	0.20 (3)	$X^2 = 5.4$ $df = 1$ $p < 0.025$
F1 hybrid: Wd X rip versus F1: rip X rip	0.67 (0.2)	$X^2 = 3.3$ $df = 1$ $p < 0.06$	0.74 (20)	$X^2 = 6.3$ $df = 1$ $p < 0.025$
F1 hybrid: Wd X rip versus F1: Wd X Wd	0.72 (28)	$X^2 = 7.4$ $df = 1$ $p < 0.01$	0.56 (9)	$X^2 = 0.3$ $df = 1$ NS
F1: Wd X Wd versus F1: rip versus rip	0.80 (41)	$X^2 = 18.8$ $df = 1$ $p < 0.0001$	0.85 (22)	$X^2 = 12.5$ $df = 1$ $p < 0.0001$

Proportion of trials (absolute number). Rip = riparian; Wd = dry woodland.

To obtain five phenotypes instead of three, we assumed incomplete dominance in the alleles for aggression and fear. Genotypes were quantitatively assigned to phenotypes by summing the following numerical values attributed to various allele combinations: AA = +5, Aa = +3, aa = +1, BB = +1, Bb = +0.75, bb = -2 (after technique used in Maynard Smith & Riechert, 1984). The phenotype, cannibal exhibited a mean sum of 5.9, hybrid of 3.9, arid-land of 3.0, riparian of 1.9, and chicken of 0. The results of this analysis are presented in Table 7. The extreme phenotypes appear primarily in the offspring produced by F2 generation hybrids and backcrosses to the parent populations (Table 7). They would be expected with introgression following the initial between-population hybridization.

For the most part, the assignment of genotypes was robust in this extension of the directional dominance model to denote the extremes of the aggressiveness continuum as unique phenotypes: there were clear numerical differences between most phenotypes based on the values assigned to different allele combinations for aggression and fear. However, this was not

the case for the female genotype Aabb (Table 7). It could have just as easily been assigned to the riparian phenotype as the chicken phenotype it was assigned to. Its numerical value is 1.00; whereas the other genotypes assigned to the phenotype chicken have a genotype value of -1.00. Genotypes assigned to the riparian phenotype had genotype values that ranged between 1.75 and 2.00 (mean = 1.9) (Table 7). We included it in the chicken phenotype because of the high representation of this phenotype in the field breeding studies.

Model of migration of arid-adapted alleles into a riparian population

Genotype frequencies were affected by the average rate of immigration from the arid-land habitat, dry woodland, with higher levels leading to lower equilibrium frequencies of the riparian genotype (Figure 4). However, within a few generations of the interruption of potential gene flow, the riparian genotype returns to unity (Figure 4). At higher levels of immigration there are marked differences between the frequencies

Table 7. Punnett square predictions for *A. aperta* aggressiveness based on underlying genotype

Parents		Phenotype	'cannibal'	'hybrid'	'arid-land'	'riparian'	'chicken'
Female	Male	Aggression level	>hybrid	>arid-land	>riparian	>chicken	<Riparian
		Genotype Value	5.9	3.9	3	1.9	0
		Genotypes:					
		female	AABB ₃ ,AABb ₇	AaBb ₂ ,AaBB ₅	AAbb ₀	aaBB ₁ ,aaBb ₈	aabb ₄ ,Aabb ₆
		male	A_BB ₄ ,A_Bb ₂		A_bb ₀	a_BB ₁ ,a_Bb ₃	a_bb ₅ ,
		Offspring					
		F1 Parentals					
rip	rip	Female				1.0	
		Male				1.0	
wd	wd	Female			1.0		
		Male			1.0		
		F1 hybrids					
rip	wd	Female		1.000			
		Male				1.000	
wd	rip	Female		1.000			
		Male	1.000				
		F2 hybrids					
wd X rip	wd X rip	Female	0.375	0.375	0.125		0.125
		Male	0.375		0.125	0.375	0.125
rip X wd	rip X wd	Female		0.375	0.0	0.375	0.250
		Male	0.375		0.125	0.375	0.125
		Backcrosses					
wd X rip	rip X wd	Female	0.250	0.250	0.250		0.250
		Male	0.250		0.250	0.250	0.250
wd X rip	rip X rip	Female		0.500		0.500	
		Male	0.500			0.500	
wd X wd	rip X wd	Female		0.500			0.500
		Male	0.500		0.500		
wd X wd	wd X rip	Female	0.500		0.500		
		Male	0.500		0.500		
rip X rip	rip X wd	Female		0.0		1.000	
		Male				1.000	
rip X rip	wd X rip	Female		1.000		0.0	
		Male				1.000	

Rip = riparian population, wd = dry woodland population. Assignment to phenotype based on summation of aggression and fear scores of genotypes, where AA = +5, Aa = +3, aa = +1, BB = +1, Bb = +0.75, bb = -2 and A = high aggression, B = low fear. Subscripts under genotypes refer to index values in recursion model.

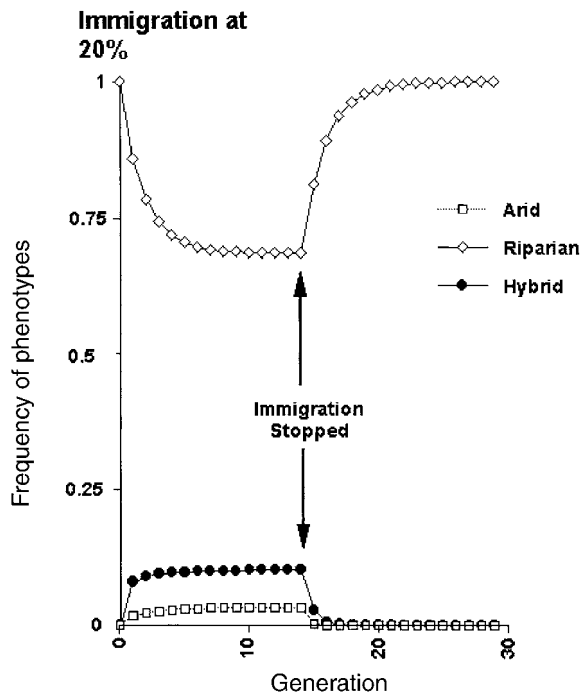


Figure 4. Dynamics of phenotype representation in riparian habitat: the initiation of immigration of arid-land spiders at a level of 20% of the population deme followed by the cessation of immigration after 15 generations. Results of a recursion equation model.

of offspring genotypes produced and those that survived to reproduce. In the absence of the immigration of arid-land immigrants, for instance, approximately 78% of the offspring in the riparian population do not survive to reproduction. This value increases to 86% when 20% of the breeding population consists of arid-land migrants (Figure 5). Because of sex-linkage in the aggression component of spider aggressiveness, the relative proportions of male and female offspring failing to survive in the riparian habitat varies with the level of immigration of arid-land spiders (Table 7). This affects the operational sex-ratio of the riparian population with the proportion of breeding males decreasing as migration rate and associated population mixing increases (Figure 6).

Discussion

Prezygotic barriers to population mixing?

Aside from hybridization (reviewed in Bullini, 1994; Wirtz, 1999), sympatric speciation in animals is now hypothesized to result primarily from initial genetic changes that lead to a shift in habitat patch use in

a heterogeneous environment (i.e., an ecological segregation model as opposed to a disruptive segregation model). Subdivision of the population started in this way is reinforced by subsequent behavioral isolation in mating (e.g., the development of habitat-based assortative mating genes (Bush, 1994)). Presumably, with chemical or morphological adaptation to the new patch type, the mixing of individuals utilizing different food sources results in sufficient gamete wastage to favor a mutation that imposes assortative mating on the system. Pickles and Grahame (1999) provide a good example of the two step evolutionary process of sympatric speciation. They found that the gastropod *Littorina saxatilis* (Olivi) shows a mating preference for shell morphs of same type in a system where there is a postzygotic barrier to local population mixing. The two morphs occupy different littoral zones and are assumed to exhibit adaptations specific to these different environments.

The best evidence for sympatric speciation in sexually reproducing animals comes from a system in which mate choice is subsumed within habitat association. The classic example comes from the frugivorous fly genus *Rhagoletis*. Species groups within this genus are morphologically similar and sympatric with each species infesting a different host (e.g., Bush, 1969, 1993). A recent example of speciation in progress is the race formation noted in the gall maker, *Erosta solidaginis* Fitch, another consequence of shifting hosts (Brown et al., 1996). Potential speciation events have also been suggested for other invertebrates that have changed habitat associations (e.g., an estuarine amphipod that has evidenced shifts in substrate association Stanhope et al., 1993). The key to genetic divergence in these particular arthropod systems is in the high degree of intimacy between the animal species and the host species or substrate it is associated with. Because mating only occurs on the chosen host plant or substrate, microhabitat preference is synonymous with mating preference in these systems. Strong habitat selection, thus, is sufficient to limit gene flow below some threshold for population differentiation to proceed. The process of speciation is greatly accelerated in these special systems because the subdivision of an existing population only requires one shift to occur, that involving the utilization of new habitat.

The *A. aperta* system as described by our study of a particular riparian and dry woodland local population interaction does not fit the model of sympatric speciation by intimate association with habitat. We know from previous study that *A. aperta* exhibiting

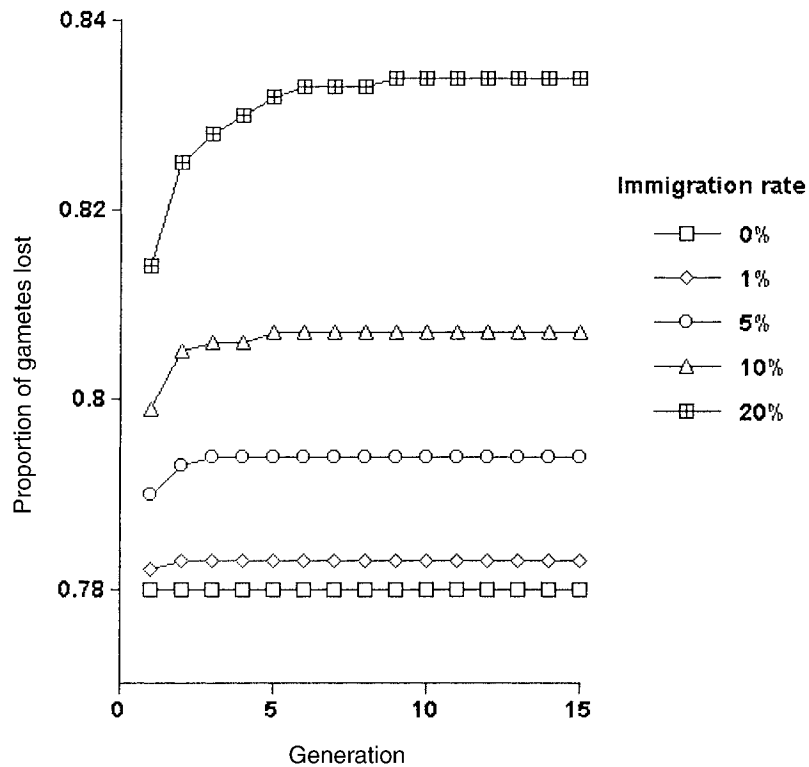


Figure 5. Predicted relationship between rate of immigration of arid-land spiders into riparian habitat (i.e., percentage of population deme) and proportion of offspring lost over time in riparian population. Results of a recursion equation model.

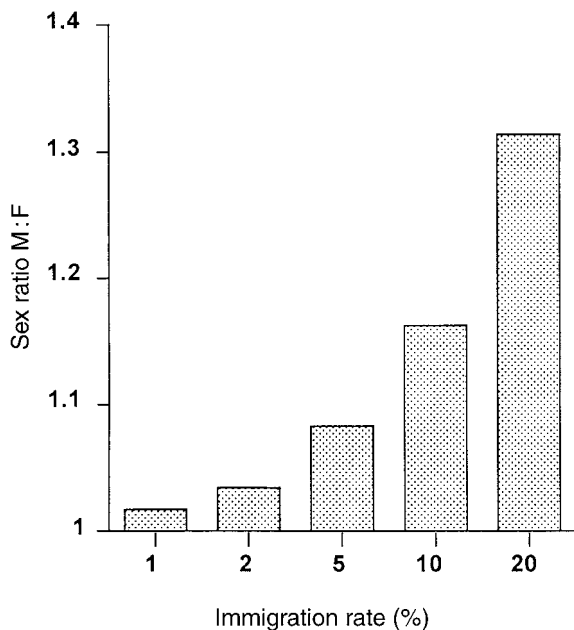


Figure 6. Predicted shift in operational sex ratio of riparian population under different levels of immigration of arid-land spiders into the riparian habitat (i.e., percentage of population deme). Result is a consequence of sex-linkage in the aggression component of spider aggressiveness.

the less aggressive/more fearful riparian phenotype do not survive to maturity in the more arid habitats (Riechert & Hall, 2000). However, arid-land adapted spiders readily move into riparian habitat and have some survival success there. In this study we found that incorporation of arid-land spiders into the riparian population deme is proportional to their rate of immigration and their mating success is proportional to their representation in the population deme. Neither habitat selection nor assortative mating barriers to population mixing appear to be in place to prevent the mixing of arid-land and riparian genotypes in riparian habitat 'islands'. Laboratory breeding studies completed on the two local populations also failed to identify a mating bias exhibited towards any phenotype by either sex.

The level of gene flow did vary, however, between the 2 years of study, which included a typical dry year and an exceptionally wet one. The influx of arid spiders into the riparian habitat was higher in the more typical drought year and the proportion of migrants engaged in matings with riparian natives showed a similar relationship. The lower availability of web sites that permit survival to reproduction in dry years in the arid habitat seems to lead to greater spider

movement in general in these years. Chance influx into the riparian area occurs as a result of the greater level of spider activity. Using a reciprocal transplant experiment, Riechert and Hall (2000) identified the selection differential against arid-land phenotypes in riparian habitat to be 0.41. Despite the disadvantage compared to riparian natives, arid-land spiders have as high a probability of survival in the riparian habitat as they would achieve in native dry woodland habitat. At this level of analysis, then, we would conclude that arid-land spiders might well be favored to migrate into riparian habitats. If they survive, their higher aggressiveness leads to greater consumption levels and greater body mass (Riechert & Hall, 2000). Spider fecundity is highly correlated with body mass (Hammerstein & Riechert, 1988; Riechert & Roeloffs, 1993; Singer & Riechert, 1995).

Postzygotic barriers to introgression

Despite the fact that arid-land spiders do not show a preference for their native habitat, gene flow is ultimately limited. The critical factor is not survival of the immigrants or some prezygotic barrier to their mating success, but rather the ultimate fitness criterion, the success of their offspring. Approximately 10% of the matings in the riparian habitat in a typical dry year involve a migrant individual from arid habitat. These matings largely produce between-population hybrids: immigrant arid-land *A. aperta* mated only with riparian natives in the 2 years of our field census of matings. From lab breeding experiments we have learned that hybrid females resulting from the mixing of the two populations are more aggressive than either parental line (agonistic behavior Maynard Smith & Riechert, 1984; size of territory demanded Riechert & Maynard Smith, 1989; aggressiveness towards prey Maupin & Riechert, 2001, in press). The selection differential against hybrids in the riparian habitat then should be even higher than the 0.5 value noted for arid-land phenotypes (Riechert & Hall, 2000). Our model of the riparian population, based on the behavioral genetics of the system, predicts a substantial decrease in the number of offspring that survive and reproduce as a result of mixing with arid-land-adapted spiders. Most of the loss occurs as a result of selection against F1 hybrids with only a small proportion of the hybrids surviving to produce F2 generation hybrids and backcross generation individuals.

Those F1 hybrids that do survive and mate produce offspring that have a fitness of 0, the cannibal and

chicken phenotypes we observed in our field censuses of the breeding system in the riparian habitat. Because of extreme aggressiveness in the former and high fear in the latter, these individuals fail to reproduce even when surviving to reproduction. This postzygotic barrier to population mixing appears to be a consequence of the genetic mechanism that determines the level of aggressiveness individual spiders exhibit in a suite of fitness linked traits that are pleiotropic effects of the same genes (Riechert & Hedrick, 1993).

Because of sex linkage in the tendency to attack/aggression component of spider aggressiveness, our model predicts that the genetic asymmetry between males and females will shift the operational sex ratio towards a predominance of females. While the predicted shift is only 5% at a migration rate of 20%, this might affect the ability of females to find mates, and thus, the relative fitness of male and female offspring (*sensu* Fisher, 1930).

The reduction in fitness we observe in hybrid *A. aperta* is an example of what is referred to as ecological postzygotic isolation (Price & Waser, 1979). In its initial form, this hypothesis proposes that the traits hybrids exhibit are intermediate to that of the respective parental types and, thus, ill suit them to the habitat occupied by either parent. Here we extend the hypothesis because the genetics of the fitness-linked behavioral system of *A. aperta* leads to hybrids that are not intermediate to the parental types but rather out of the bounds of either type. One might even conclude that there is further a genetic postzygotic isolation evidenced in the failure of some backcross and F2 generation individuals to breed (Rice & Hostert, 1993). The break up of favorable gene combinations that have positive epistatic interactions in the parent species may underlie the production of the extreme phenotypes noted in the riparian system we studied (see discussion of genetic mechanism below). While Schluter (1998) views these ecological and genetic isolating mechanisms to be independent and proposes tests that will distinguish between them, the *A. aperta* system suggests that these two modes of isolation need not be independent. Rather the genetic isolating mechanism may both pose postzygotic consequences independent of environment and at the same time result from environmental-induced selection against hybrids in the parental habitats.

The genetic model for spider aggressiveness

The results of this study, in general, support the initial model Maynard Smith and Riechert (1984)

proposed to explain the behavior of individuals representing two *A. aperta* populations and hybrids between them. The initial model, which assumed the existence of directional dominance in genes underlying two components to aggressiveness, was applied to populations separated by approximately 600k. We found this same model explains the behavior of individuals from adjacent local populations occupying arid versus riparian habitats as well as the behavior of their F1 hybrid offspring. Our studies have provided further insight into the model as structured by Maynard Smith and Riechert (1984). It has been necessary to assume that there is some intermediate expression of dominant alleles to explain the behavior of the extreme phenotypes discussed above. Note that Maynard Smith and Riechert (1984) referred to the aggression and fear components of spider aggressiveness as genes or gene complexes. Epistasis of multiple genes at different loci in a gene complex could also produce the intermediacy we prescribe for heterozygotes.

Instead of the three phenotypes identified in the initial model, the assumption of intermediacy in allele expression produces five discernible ones exhibiting a dominance ranking based on level of aggressiveness of: cannibal > hybrid > arid-land > riparian > chicken (Females exhibit nine potential genotypes and males six). While simple, this genetic model explains the outcomes of mixing between arid-land and riparian phenotypes remarkably well. Only the assignment of male F1 hybrids who receive their sex chromosome (aggression component of aggressiveness) from a female of arid-land phenotype to the cannibal phenotype seems problematic. Numerically, A_Bb with a genotype value of 5.75 is closely aligned to the female cannibal genotypes, which exhibit a mean genotype value of 5.9. Males from this cross, however, score behaviorally more like arid-land phenotypes than cannibals (e.g., agonistic behavior trial results Table 6).

This may reflect the fact that the aggression component of aggressiveness is the dominant factor in determining contest outcome. However, another genetic study completed on the behavior of *A. aperta* aligned this male class with the hybrid phenotype (i.e., in their level of superfluous killing of prey Maupin & Riechert, in press). Breeding experiments explicitly designed to test the levels of aggression exhibited by cannibals produced through various crosses are needed to clarify the position of this male hybrid class.

The speciation process

In summary, the two local populations experience different selection pressures and have differentiated behaviorally in response to their respective selective environments. Without gene flow these populations may quickly diverge to become locally adapted as indicated by the enclosure experiment presented in Riechert (1993a), which detected a significant shift in phenotype after one generation of selection in the absence of gene flow. Our model results further predict that within three generations of the cessation of gene flow, the riparian population would be free of mixed genotypes because of the high selection coefficients operating against immigrants and hybrids. If gene flow exists between two populations, each may not be as adapted to its local environment as a similar population that experiences no gene flow would be. While there has been considerable theoretical debate over whether gene flow can limit adaptation (e.g., Barton & Whitlock, 1997), this is one of the first empirical studies to have tested the hypothesis that gene flow limits adaptation. It appears to do so in this system.

It appears as if two evolutionary pathways might eliminate the high levels of gamete wastage that presently occur. One would expect that a discrimination allele that led to assortative mating with respect to behavioral phenotype would rapidly spread in a riparian habitat and lead to further population differentiation. This would be a behavioral isolating barrier every bit as strong as a geographic or physical habitat barrier to gene flow. On the other hand, a flexibility allele might also permit individuals to perform equally well in both riparian islands and the surrounding matrix of more arid habitats. Aggressiveness, in this case, would be context dependent and the population panmictic. The assortative mating pathway would lead to a speciation event, while the flexibility pathway would not. Rather it would make the species even more of a habitat generalist than it already is.

There is a third alternative pathway, one in which there is selection for modification of expression. Such modification would be particularly important to the cannibal and chicken phenotypes. The three alternatives differ in the degree to which they might be favored by selection. This, in part, depends on the rate of gene flow and on the presence of sufficient variability for selection to operate on. Of the three alternatives, modification of expression might be the easiest to obtain but would offer the least fitness gain.

While the local populations of *A. aperta* used in this study have not embarked on one of these evolutionary pathways, this species exists as a very extensive metapopulation (in the sense of Hastings & Harrison, 1994). The arid-land habitat favoring an aggressive phenotype extends from Wyoming to central Mexico and from inland California to central Texas. Embedded within this framework are hundreds of springs, streams, and rivers that support riparian habitat patches that differ in extent and shape. The *A. aperta* metapopulation is ideal for the completion of a chronosequential analysis of population divergence in which change through time is mirrored in space. We hope to locate populations that are in different stages of divergence and to explain this divergence in future studies, which will include detailed analyses of local adaptation with phylogeographical analyses of historical processes (Avise et al., 1987; Templeton et al., 1995; Avise, 1998).

Acknowledgements

This work was supported by grants from the Population Biology and Animal Behavior Programs at NSF. Much of the fieldwork was completed on the grounds of the American Museum of Natural History's Southwestern Field Station. We would like to thank the field station staff, their volunteers and other researchers on site who helped with the construction and maintenance of the enclosure and with the censusing. Special thanks to Rikki Hall, Philip Johns, Jen Maupin, Barbara Roth, and Sharman Wisdom for their help in completion of the fieldwork.

References

- Avise, J., 1998. The history and purview of phylogeography: a personal reflection. *Mol. Ecol.* 7: 371–379.
- Avise, J., J. Arnold, R.M. Ball, E. Bermingham, T. Lamb, J. Neigel, C. Reeb & N. Saunders, 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annu. Rev. Ecol. Syst.* 18: 489–522.
- Barracough, T.G. & A.P. Vogler, 2000. Detecting the geographical pattern of speciation from species-level phylogenies. *Am. Natur.* 155: 419–434.
- Barton, N. & M. Whitlock, 1997. The evolution of metapopulations, pp. 183–214 in *Metapopulation Biology, Ecology, Genetics and Evolution*, edited by I. Hanski & M.E. Gilpin. Academic Press, San Diego.
- Brown, J.M., W.G. Abrahamson & P.A. Way, 1996. Mitochondrial DNA phylogeography of host races of the goldenrod ball gall maker, *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution* 50: 777–786.
- Bullini, L., 1994. Origin and evolution of animal hybrid species. *TREE* 9: 422–426.
- Bush, G.L., 1969. Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera: Tephritidae). *Evolution* 23: 237–251.
- Bush, G.L., 1993. Host race formation and speciation in *Rhagoletis* fruit flies (Diptera: Tephritidae). *Psyche* 99: 335–357.
- Bush, G.L., 1994. Sympatric speciation in animals: new wine in old bottles. *TREE* 9: 285–288.
- Fisher, R.A., 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Hammerstein, P. & S.E. Riechert, 1988. Payoffs and strategies in spider territorial contests: ESS-analyses of two ecotypes. *Evol. Ecol.* 2: 115–138.
- Hastings, A. & S. Harrison, 1994. Metapopulation dynamics and genetics. *Ann. Rev. Ecol. Syst.* 25: 167–188.
- Lowe, C.H., 1964. *The Vertebrates of Arizona*. University of Arizona Press, Tucson, AZ.
- Maupin, J.L. & S.E. Riechert, Superfluous killing in spiders: a consequence of adaptation to food-limited environments? *Behav. Ecol.* (in Press).
- Maynard Smith, J. & S.E. Riechert, 1984. A conflicting tendency model of spider agonistic behaviour: hybrid-pure population line comparisons. *Anim. Behav.* 32: 564–578.
- Mayr, E., 1988. *Towards a New Philosophy of Biology: Observations of an Evolutionist*. Harvard University Press.
- Papke, M., S.E. Riechert & S. Schulz, 2001. An airborne spider pheromone associated with male attraction and release of courtship. *Anim. Behav.* 61: 1–11.
- Pickles, A.R. & J. Grahame, 1999. Mate choice in divergent morphs of the gastropod mollusc *Littorina saxatilis* (Olivi): speciation in action? *Anim. Behav.* 58: 181–184.
- Price, M.V. & N.M. Waser, 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. *Nature* 277: 294–297.
- Rice, W.R. & E.E. Hostert, 1993. Laboratory experiments on speciation: what we have learned in 40 years? *Evolution* 47: 1637–1653.
- Riechert, S.E., 1979. Games spiders play II: resource assessment strategies. *Behav. Ecol. Sociobiol.* 4: 1–8.
- Riechert, S.E., 1981. The consequences of being territorial: spiders, a case study. *Am. Natur.* 117: 871–892.
- Riechert, S.E., 1987. Between population variation in spider territorial behavior: hybrid-pure population line comparisons, pp. 33–42 in *Evolutionary Genetics of Invertebrate Behavior*, edited by M. Huettel. Plenum Press, New Jersey.
- Riechert, S.E., 1993a. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. *Behav. Ecol. Sociobiol.* 32: 355–363.
- Riechert, S.E., 1993b. A test for phylogenetic constraints on behavioral adaptation in a spider system. *Behav. Ecol. Sociobiol.* 32: 343–348.
- Riechert, S.E., 1999. Using behavioral ecotypes to study evolutionary processes, pp. 3–32 in *Geographic Variation in Behavior: Perspectives on Evolutionary Mechanisms*, edited by S. Foster & J. Endler. Oxford University Press, Oxford.
- Riechert, S.E. & R.F. Hall, 2000. Local population success in heterogeneous habitats: reciprocal transplant experiments completed on a desert spider. *J. Evol. Biol.* 13: 1–10.
- Riechert, S.E. & A.V. Hedrick, 1990. Levels of predation and genetically based anti-predatory behavior in the spider, *Agelenopsis aperta*. *Anim. Behav.* 40: 679–687.
- Riechert, S.E. & A.V. Hedrick, 1993. A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta* (Araneae: Agelenidae). *Anim. Behav.* 46: 669–675.

- Riechert, S.E. & R.M. Roeloffs, 1993. Inbreeding and its consequences in the social spiders, pp. 283–303 in *The Natural History of Inbreeding and Outbreeding*, edited by N. Thornhill. University of Chicago Press, Chicago.
- Riechert, S.E. & J. Maynard Smith, 1989. Genetic analyses of two behavioural traits linked to individual fitness in the desert spider, *Agelenopsis aperta*. *Anim. Behav.* 37: 624–637.
- Riechert, S.E. & F. Singer, 1995. Investigation of potential male mate choice in a monogamous spider. *Anim. Behav.* 49: 715–723.
- Riechert, S.E. & C.R. Tracy, 1975. Thermal balance and prey availability: bases for a model relating web site characteristics to spider reproductive success. *Ecology* 56: 265–284.
- Schluter, D., 1998. Ecological causes of speciation, pp. 114–129 in *Endless Forms: Species and Speciation*, edited by D.J. Howard & S.H. Berlocher. Oxford University Press, Oxford.
- Singer, F. & S.E. Riechert, 1995. Mating system and mating success in the desert spider, *Agelenopsis aperta*. *Behav. Ecol. Soc.* 36: 313–322.
- Singer, F., S.E. Riechert, H. Xu, A.W. Morris, E. Becker, J.A. Hale & M.A. Nouredine, 2000. Analysis of courtship success in the funnel-web spider *Agelenopsis aperta*. *Behav.* 137: 93–117.
- Smiley, T.L., J.D. Nations, T.L. Pewe & E.P. Schafer, 1984. *Landscapes of Arizona: The Geological Story*. University Press of America, Lanham, MD.
- Stanhope, M.J., B. Hartwick & D. Baille, 1993. Molecular phylogenetic evidence for multiple shifts in habitat preference in the diversification of an amphipod species. *Mol. Ecol.* 2: 99–112.
- Templeton, A.R., E. Routman & C.A. Philips, 1995. Separating population structure from population history: a cladistic analysis of the geographic distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. *Genetics* 140: 767–782.
- Wirtz, P., 1999. Mother species-father species: unidirectional hybridization in animals with female choice. *Anim. Behav.* 58: 1–12.