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Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider

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Summary. This study investigates the possibility that gene flow underlies the apparently maladaptive behavior of a riparian woodland population of the desert spider *Agelenopsis aperta* with respect to territorial, foraging, and antipredatory behaviors. I found that other local populations of *A. aperta* in the vicinity of the riparian woodland habitat are prey-limited and exhibit an "aridlands" phenotype (high aggressiveness in competitive interactions over energy-based territories and a lack of discrimination among potential prey types). The riparian woodland population deviates from surrounding populations in the area in that prey are abundant and this population shows a mixture of "aridlands" and "riparian" (low aggressiveness towards conspecifics and discrimination of prey profitability) phenotypes. Electrophoretic analyses of population subdivision in the area indicate that significant levels of gene flow have occurred, at least, sometime in the past. Drift fence analyses of spider movement futher indicate that there is marked unidirectional movement of spiders each year from the more arid habitats into the riparian woodland. Experimental manipulation of gene flow and predation pressure demonstrates that gene flow restricts adaptation in this habitat: one generation of predation pressure in the absence of gene flow is sufficient to cause a marked shift in spider behavior towards the expected "riparian" phenotype.

Introduction

The idea that animals are maximizers of one sort or another because natural selection has made them so is a basic tenet of behavioral ecology (Grafen 1991). However, systematic investigation of the fitness consequences of certain traits sometimes leads to the falsification of the hypothesis that these patterns are adaptive (e.g. Cheverud et al. 1985; Wanntorp et al. 1990; Via 1991). Factors that can lead to maladaptation include conflict between sexual selection and natural selection (e.g., the classic case of the giant antlers of Irish elk: Maynard Smith 1978) genetic system constraints (Lewontin 1987), fluctuating selection pressures or recency of change (Maynard Smith 1978; Krebs and Davies 1981), phylogenetic constraints (Brooks and Wiley 1988), and gene flow (Slatkin 1985a).

I have been working with behavioral traits in a spider species, *Agelenopsis aperta* (Gertsch), that adapt individuals to different selective environments. These traits show between-population differences that have genetic bases (Maynard Smith and Riechert 1984; Riechert and Maynard Smith 1989; Hedrick and Riechert 1989; Riechert and Hedrick 1990) and thus may be subject to selection. *Agelenopsis aperta* is an annual spider species that builds a sheet web with an attached funnel. The spider sits within the protected environment of the funnel when not foraging or engaging in territorial interactions. *Agelenopsis aperta* occupies predominantly aridland habitats from northern Wyoming to southern Mexico and from California to central Texas.

During the course of my investigation of behavioral adaptation in local populations of *A. aperta*, I have identified one population that fails to be at adaptive equilibrium with respect to the several traits that I have investigated [i.e., territory size (Riechert 1981), agonistic behavior (Hammerstein and Riechert 1988), diet choice (Riechert 1991), and anti-predator behavior (Riechert and Hedrick 1990)]. This population exists under unusually ameliorated conditions, where its prey are abundant, but it also has higher levels of bird predation than most other species populations of *A. aperta*.

I have falsified all of the potential limiting factors that might explain the observed deviation of the population with the exception of gene flow [see Riechert (1993a) for review]. Briefly, S.E. Riechert and F.D. Singer (in preparation) found that the body masses male *A. aperta* gain as territorial animals throughout most of their lifespans influences male reproductive success, in that larger males can travel further in search of matings, live longer following maturity, outcompete other males for females and are more likely to be accepted by females. Natural selection and sexual selection pressures are thus similar in this species. The hypothesis that genetic constraints prevent adaptation (i.e., phylogenetic inertia) is falsified in the companion paper to this one (Riechert 1993b). I found that a population of *A. aperta* that exists under similar selection pressures to the population under investigation, but that lacks the potential for gene flow from surrounding more rigorous habitats, exhibits the behavior patterns predicted for a population exposed to abundant food and predation pressure by birds. Recency of change is eliminated from consideration, because the habitat is supported by spring-fed streams that have been in existence for hundreds of years. Finally, I have been studying the population since 1969, and have not observed significant fluctuations in prey availability to *A. aperta* in the area (Riechert 1991). Further, these deviations from expected behavior are observed year after year, regardless of the conditions present in any given year (e.g., drought versus wet years). These results have led me to consider the possible role of gene flow in limiting adaptation to local conditions.

The potential interaction between gene flow and selection is a matter of scale. Whereas some species may be divisible into discrete breeding units, others may be more cohesive. The view of Van Valen (1976) that demes are ecologically or selectively homogeneous does not necessarily hold, because genetic neighborhoods may indeed be larger than the selectively homogeneous environments. Brandon (1990) feels experimental work is needed that considers this potential problem of scale.

The study reported here considers: (1) the possibility that gene flow is occurring among local populations of the spider *Agelenopsis aperta* in a desert mountain range in southeastern Arizona (AZ); and (2) the extent to which gene flow might limit adaptation in an unusually ameliorated habitat for this spider species, AZ riparian woodland. The majority of the habitats available to this spider in the area are aridlands. Prior experience indicates that such habitats afford *A. aperta* limited prey availabilities and limited sites that provide protection from thermal extremes (Riechert 1978). The "aridland" behavioral phenotype is one that is aggressive in territorial disputes (Hammerstein and Riechert 1988) and that attacks most insect types that it encounters (Riechert 1991).

The population that fails to exhibit behavior patterns that are adaptive occupies an unusually mesic environment within the area, a riparian woodland that is supported by a permanent stream. Prey levels are high and web-sites abundant (Riechert 1981). This habitat is also favorable to birds and they exert strong predation pressure on the degree of timorousness riparian woodland *A. aperta* should exhibit in response to predatory cues, a selection pressure that is insignificant in the more arid environments (Riechert and Hedrick 1990). Levels of aggression versus fear or some similar scalar parameters (e.g., tendency to attack or flee) seem to underlie the behavioral traits that adjust the behavior of *A. aperta* to different local environments: territory size, level of aggressiveness in fighting over web-sites and associated territories, timorousness to predatory cues, and latency of prey attack. These behavior patterns have been shown to be phenotypically correlated at both the individual and population levels (Riechert and Hedrick in press). Riparian woodland *A. aperta* deviate from predicted behavior in all of these traits. Because prey levels are very high in the riparian woodland habitat, *A. aperta* there are not predicted to be territorial [i.e., defend territories in excess of their webs (Riechert 1991)]. They do, however, exhibit territorial behavior (Riechert 1981). Riparian woodland *A. aperta* are predicted to withdraw from disputes over webs when they are the smaller opponent and to restrict contest behavior to vibratory and visual displays when they are the larger opponent (Hammerstein and Riechert 1988). Hammerstein and Riechert observed unexpectedly high frequencies of escalation to physical contact and fighting in this spider population. Riparian woodland spiders attack a broad spectrum of prey which include prey types that (1) requires long handling time (exposing the spiders to bird predation on the web-sheet) and (2) can cause injury (Riechert 1991). Prey levels are sufficiently high in the habitat that this spider is predicted to specialize on those prey types that require little handling time and afford no risk of injury (Riechert 1991). Riparian woodland spiders on average show a higher probability of retreat into the protection of the web-funnel when presented with a predatory cue than aridlands spiders. However, there is much unexpected individual variation in this trait (Riechert and Hedrick 1990).

In this study I consider the degree to which the selective environment changes with habitat in the Chiricahua Mountain area through measurements of prey availabilities. I also compare the competitive and foraging behaviors of individual *A. aperta* occupying the different habitats in the area. I then consider the degree to which the *A. aperta* population in the area is genetically subdivided, using electrophoretic analyses of population genetic structure as an indirect measure of gene flow, as well as drift fence assessment of spider migration. Finally, I report on a field experiment that delineates the role of gene flow in limiting behavioral adaptation to strong bird predation pressure and abundant prey in the riparian woodland habitat.

Methods

Study areas

My work has centered on two populations located in the south-central part of this range. One inhabits a desert grassland area in south-central New Mexico (NM) that offers daily temperature extremes, high winds, and limited prey. The other inhabits a riparian woodland area in southeastern Arizona (AZ), where temperatures are moderated by a tree canopy and permanent stream, and where potential insect prey are abundant. More detail concerning the different environments afforded by these two sites is provided in Riechert (1993b).

Among-habitat variation in selection pressures and spider behavior in the Portal, Arizona area

To investigate potential gene flow influences on the behavior exhibited by riparian woodland spiders, I extended the study to other

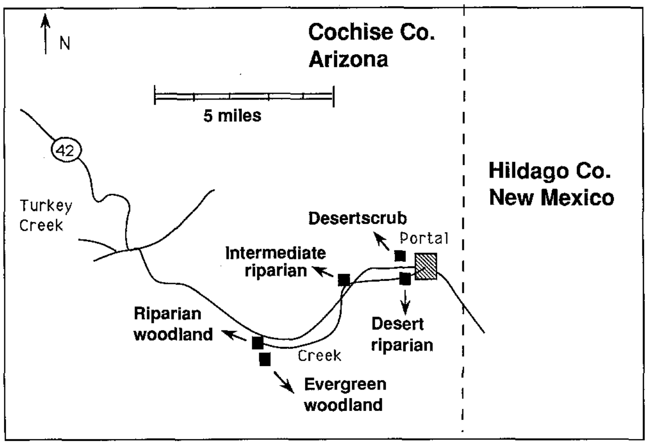

Fig. 1. Positions of various *Agenelopsis aperta* local populations in Chiricahua Mountain area of southeastern Arizona

local populations of *A. aperta* in the Portal, Arizona area (Fig. 1): those occupying evergreen woodland, desert riparian and southwestern desertscrub habitats (classification of Lowe (1964)). The AZ riparian woodland habitat is a narrow strip (approximately 50 m in width) of flat land bisected by Middlefork Creek. As one leaves the influence of this waterway and moves up the slopes, the riparian woodland is replaced by an open, evergreen woodland dominated by various evergreen oak species (*Quercus emoryi* is the most common). As one follows the creek downslope towards the desert flats, it becomes the intermittent South Fork Creek and supports only a desert riparian habitat that is dominated by mesquite (*Prosopis juliflora*). Beyond the influence of the creek bed in this area the habitat is southwestern desert-scrub and is dominated by arid-adapted shrubs and cacti. These are sparsely distributed along with patches of grass on a gravel substrate.

Prey availability. I used the sticky trap protocol described in Riechert (1993b) to assess prey availabilities to *A. aperta* in each of these habitats. The sampling was completed in the same field season (June–July), a period coinciding with the maturation and egg mass production of *Agelenopsis aperta.*

Behavior. Two behavioral measures were studied on samples of spiders from each habitat: contest behavior and diet choice. The first indicates the level of competition for web-sites that exists in a given habitat, while the second measure provides an estimate of the feeding levels offered by the habitat (in an evolutionary rather than in an immediate sense since diet choice has a genetic basis in this species: Hedrick and Riechert 1989).

Contests. I flagged 80 web-sites within each habitat and subsequently collected, weighed and individually paint-marked the web occupants at the flagged sites. (I used a fast-drying enamel paint on the dorsum of the abdomen.) I then used the contest protocol described in Riechert (1993b) to obtain as many records of contest behavior as weight matches could be found. Since each intruder and each owner were used only once in the sampling, contest number varied with the number of weight matches that could be made between designated territory owners and intruders in the respective habitats.

Diet choice. Prey types with high (8.3–25.0 j/s capture effort) versus low (0.2–0.6 j/s) profitabilities (Riechert 1991) were offered to spiders in each habitat. The implication is that spiders in a food-rich environment will, at least, show lower attack rates towards the low profitability prey, while spiders in food limited environments will show a high attack rate towards all prey (Pyke 1984). At least 25 prey of each profitability category were offered to spiders in each habitat, using the protocol described in Riechert (1993b).

Tests for gene flow among local populations

I utilized two approaches in determining the potential influence of gene flow on riparian woodland spider behavior: (1) electrophoretic analyses of local population genetic structure, and (2) drift-fence monitoring of spider movement between the riparian woodland habitat and adjacent evergreen woodland (Fig. 1).

Electrophoretic analyses. I collected a minimum of 20 gravid females from each of five local AZ populations: riparian woodland, evergreen woodland, desert riparian, desertscrub and a habitat that was halfway between riparian woodland and desert riparian on the same creek (Fig. 1). Upon collection, gravid females tend to lay their egg cases. I thus did not prepare the females for processing until they had laid these cases. The eggs were returned to the respective study areas and I then put the females in liquid nitrogen for return to the laboratory and subsequent processing.

Whole individual spiders were utilized in the electrophoretic analyses. Each spider was ground in 1:1 (weight:volume) cold grinding buffer using a glass rod and porcelain spot plates. The ground tissue and buffer were collected in capillary tubes and centrifuged, and the supernatant was used for starch-gel electrophoresis. The procedures for preparing gels, applying samples to the gels, and running and staining gels follow those of Selander et al. (1971) and Harris and Hopkinson (1978), with the exceptions noted in McCracken and Wilkinson (1988). The samples were systematically assayed at 17 loci: malate dehydrogenase 1 and 2 (MDH), esterase 1 and 2 (EST), lactate dehydrogenase (LDH), superoxide dismutase 1 and 2 (SOD), isocitrate dehydrogenase (IDH), fumarase 1 and 2 (FUM), α-glycerophosphate dehydrogenase (αGPD), glutamicoxaloacetic transaminase 1 and 2 (GOT), α-*n*-acetylglucosaminidase (HEX), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and galactosaminidase (GAM). Of these enzymes 14 were polymorphic, reflecting my choice of systems that could provide maximum information: only EST1, FUM1 and IPO2 were monomorphic.

Two analyses were completed on the electrophoretic data. First I used Biosys-1 (Swofford and Selander 1981) to calculate F_{ST}s. F statistics are related to amounts of heterozygosity. F_{ST} specifically is referred to as the fixation index: it is the probability that two alleles chosen at random from within the same subpopulation are identical by descent. The Nm estimate of gene flow in the general vicinity of the Chiricahua Mountains was then calculated, using the equation $Nm_{est}=(1/F_{st}-1)/4$ (Wright 1931; Reynolds et al. 1983), where N= effective population number and m= the effective proportion of immigrants (Wright 1931). I determined the extent to which the level of gene flow between the riparian woodland and other local populations varies with distance by applying Slatkin's (1985b) private allele estimate of Nm, first to all five local populations and then to successively smaller subsets of the local populations; for each successive reiteration, I deleted the most distant population. I used the following equation in the analysis:

$$\ln p(1)=a\ln(Nm)+b$$

where $p(1)$= the average allele frequency over all loci of alleles observed in only one population, and a and b are constants equal to -0.505 and -2.44 respectively for sample sizes of 25. I used the correction $Nm^{*}=Nm\times N/25$ to accommodate sample sizes that deviate from 25 (after Slatkin 1985b). Note that I chose to use the private allele technique rather than spatial autocorrelation (Sokal and Oden 1978) which would provide a similar test, because the use of spatial autocorrelation for population genetics applications is in question [see review by Slatkin and Arter (1991)].

Spider movement. I used drift fences to monitor spider movement between the AZ riparian woodland and evergreen woodland habitats over 5 weeks coinciding with the movement of male *A. aperta* in search of matings in the Portal area (July–August). I erected six 2-m long sheet-metal flashing drift fences (14 cm in height) at random locations within each of the habitats. I erected another six at the border between the two habitats, three at the base of the hill separating them (the riparian woodland edge) and three at the top of the hill (the evergreen woodland edge). Along both riparian woodland and evergreen woodland sides of the flashing wall, I placed 1-l pitfall traps at 50-cm intervals. I filled these partially with ethylene glycol and protected them from rainfall by extending sheet metal awnings out from the flashing walls. When *A. aperta* encounters a barrier, it turns and walks along it. Hence, the drift fences greatly increased the sampling area over what I might have obtained through the use of pitfalls alone. I checked the pitfalls each week for captures.

Experimental test for gene flow effects

I tested the hypothesis that gene flow restricts *A. aperta's* adaptive response to both high prey levels and high predation pressure in the AZ riparian woodland habitat by a selection experiment in the absence of gene flow. Specifically, I compared spider behavior over time in the habitat from four 4-m^2 enclosures covered with bird netting (protected treatment) with that of individuals maintained in another four enclosures that were left uncovered (exposed treatment). Position of the treatments within the study area were randomized with respect to each other.

In late fall, I removed all *A. aperta* naturally located in the enclosures and then inoculated each one with a total of 76 *A. aperta* spiderlings, such that each enclosure contained the same familial representation of individuals hatched from ten egg cases collected in this habitat. (I divided each of the ten egg cases into eight groups of equal numbers of spiderlings). I cleared the vegetation along a 50-cm wide swath of ground along the peripheries of the 30-cm high flashing enclosures to prevent spider immigration, hence potential gene flow. I left the vegetation structure within the enclosures undisturbed, permitting the system within to reach equilibrium densities through emigration.

After winter dormancy, the spiderlings were subjected in spring and summer to different selection regimes: (1) the normally high predation by birds (Riechert and Hedrick 1990) (exposed treatment); and (2) no predation by birds (protected treatment). In late summer, I counted the spiders remaining in each enclosure and ran two behavioral trials (predator cue and contest) that would show the prevalence of aridlands versus riparian phenotypes. [Remember, the "aridlands" phenotype is one that is aggressive in web-site disputes and that quickly resumes foraging following a disturbance (e.g., desert-scrub, desert riparian and evergreen woodland *A. aperta*). This is because aridlands *A. aperta* need to obtain and keep sites that provide high feeding levels and must maximize the time spent foraging. The "riparian" phenotype is one that is non-aggressive in disputes because good web-sites are abundant. It also shows greater fear towards disturbances (potential predation events)].

Behavioral tests: predator cue test. I used the bulb portion of a camera-cleaning brush to simulate the large amplitude web vibrations that an approaching bird might make on *A. aperta* webs, after methods described in Riechert and Hedrick (1990). Briefly, I directed four puffs from the bulb at 15 spiders selected at random in each enclosure as each sat in a foraging mode (facing out of its funnel with legs spread). I held the bulb at a 45° angle and at 8 cm from the funnel entrance when delivering the puffs. I scored each spider according to which puff caused it to retreat, if any, and the time it took for the individual that did retreat to return to the funnel entrance. Note that I scored a failure to retreat as 0 s latency to return to a foraging mode and that I terminated all trials after 600 s. I assigned a score of 600 s to those spiders that failed to return to their funnel entrances within this time limit.

Contest trials. I collected all spiders sighted in the enclosures and marked their respective web locations for later release. I maintained them in the laboratory in separate boxes with *ad libitum* prey levels for 3 days and then weighed them. Pairs of individuals from different treatments but equal in body mass were then individually paint-marked and released onto a web produced by a third individual that had been removed prior to the start of the trial. I placed the plastic box housing the web in a larger plastic box and covered this latter box to prevent escape of the contestants. Thus, I was able to retrieve the losing contestant following its retreat from the web without physically watching each contest to its completion.

I periodically checked each set of boxes to establish whether the individual from the exposed or protected treatment had won the trial (i.e., had ownership of the web). If both spiders were found to be in the larger box, the trial was scored as a draw. (I did not observe any pair of spiders during the course of the trials cohabiting in the web in the smaller box.) After the trials, I returned all spiders to their respective web-sites.

The next spring, I collected all *A. aperta* spiderlings present in each enclosure. This sample represents offspring of the individuals subjected to selection (*A. aperta* is an annual species). Because I collected the spiderlings at the time of their emergence from egg cases, these individuals had not experienced the imposed selection regimes themselves. I individually reared these spiders in the laboratory with food *ad libitum* through summer and then gave them the two behavioral tests described above.

Results

Among-habitat variation in selection pressure and spider behavior

Prey availability. Figure 2 shows the prey levels available to *A. aperta* for different habitat types in the Portal, Arizona area. Included for reference is NM desert grassland. Each of the drier AZ habitats offered significantly fewer trap-days of optimal prey levels (20 mg dry weight of prey/day: Riechert 1978) than did the AZ riparian

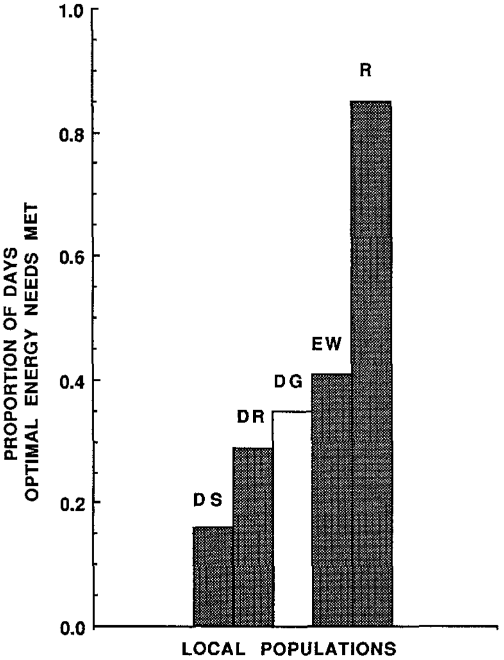

Fig. 2. Estimated prey availabilities experienced by *A. aperta* at different localities: *DS* = desertscrub, *DR* = desert riparian, *DG* = desert grassland, *EW* = evergreen woodland and *R* = riparian woodland. *Solid columns*, Arizona (AZ); *blank columns*, New Mexico (NM)

Table 1. Comparison of contest and diet choice behaviors among individuals sampled from habitats that vary in the prey availabilities they afford

	Contest behavior: probability of owner escalation[a]		Attack rates towards prey profitability			
	n	P	n	Low	n	High
Desertscrub	34	0.79	92	0.96	59	0.90
NM desert grassland	23	0.77	113	0.77	105	0.91
Desert riparian	42	0.68	118	0.62	62	0.77
Evergreen woodland	42	0.68	259	0.89	47	0.88
Riparian woodland	73	0.43	212	0.55	65	0.51

[a] Contests involving individuals that were equal in body mass

All habitats are local AZ except NM desert grassland. Contest data presented for NM desert grassland and AZ riparian woodland collected in conjunction with Hammerstein and Riechert (1988); attack rates towards high and low profitability prey for these habitats from Riechert (1991)

woodland habitat (G-test: $P<0.001$ in all comparisons). The desertscrub habitat afforded significantly fewer optimal trap days than did NM grassland (G-test: $P<0.001$), while desert riparian and evergreen woodland habitats afforded similar prey levels to those in the NM desert grassland (G-tests: $P>0.10$ in both cases).

Spider behavior. Between habitat comparisons of spider contest and diet choice behaviors are presented in Table 1. The contest parameter I used to assess the level of aggressiveness present in each local population was the contest "decision" made by the owner of the territory following the assessment phase of the contest. Individual spiders may "withdraw" from the contest following assessment of relative weight, they may "display only" (non-injurious behavior patterns), or they may "escalate" to potentially injurious behavior. Game-theoretic analyses of the *A. aperta* system by Hammerstein and Riechert (1988) indicate that where competition for web-sites is high, escalation is predicted; it is not predicted for the AZ riparian woodland habitat where food levels are high and web-sites readily abundant. Results of χ^2 tests indicate that while riparian-woodland animals did show the escalation strategy, it was observed with a lower frequency in the contests than for the other populations (Table 2a).

All of the more arid AZ populations and the NM desert grassland population showed significantly higher attack rates than riparian woodland spiders towards high-quality prey – those that provide the greatest reward compared to capture effort (Tables 1 and 2b). Between-habitat comparisons of spider behavior towards the lower-quality prey showed a more complex pattern. There were three feeding levels exhibited, with desertscrub and evergreen woodland spiders showing very high attack rates towards low profitability prey, NM desert grassland spiders showing intermediate levels of attack, and desert riparian and riparian woodland spiders showing the lowest attack rates (Table 2c). Note that optimal foraging theory predicts that riparian woodland spiders would exhibit high attack rates towards high-profitabili-

Table 2. Results of χ^2 tests (χ^2, P) for between-habitat comparisons

a) Frequency of owner choice of the strategy "escalate" in contests where the individuals are matched for weight

	Grassland	Desert	Evergreen	Riparian
Desertscrub	0.05, >0.1	0.58, >0.1	0.58, >0.1	11.31, <0.001
NM grassland		0.25, >0.1	0.25, >0.1	7.59, <0.01
Desert riparian			0.06, >0.1	6.50, <0.02
Evergreen				6.50, <0.02

b) Attack rates towards prey types of high profitability (joules obtained/capture effort = 8.3–25.0)

	Grassland	Desert	Evergreen	Riparian
Desertscrub	0.09, >0.1	1.22, >0.1	0.02, >0.1	10.90, <0.001
NM grassland		2.26, >0.1	0.00, >0.1	17.57, <0.001
Desert riparian			0.54, >0.1	4.52, <0.05
Evergreen				7.85, <0.01

c) Attack rates towards prey of low profitability (joules obtained/capture effort = 0.2–0.6)

	Grassland	Desert	Evergreen	Riparian
Desertscrub	7.29, <0.01	17.12, <0.001	1.31, >0.1	26.60, <0.001
NM grassland		4.63, <0.05	5.72, <0.02	13.70, <0.001
Desert riparian			20.87, <0.001	0.55, >0.1
Evergreen				42.66, <0.001

Table 3. *Nm* estimate of gene flow among 5 local populations in Chiricahua Mountains of southeastern Arizona (Fig. 1), where N= effective population size and m=effective proportion of immigrants. At least 20 individuals from each local population were scored for each locus. *Nm*: (a) F_{ST} estimate of general levels of gene flow (Wright 1931) in the area; (b) private allele estimates of *Nm* (Slatkin 1985b) as a function of distance from the riparian woodland habitat. $P(1)$=average frequency of alleles observed in only one population, $Nm^*=Nm$ estimate corrected for deviation of sample size from 25 and decreasing number of populations reflects the exclusion of more distant local populations from the riparian woodland (Fig. 1)

a)

Locus	F_{ST}	*Nm*
PGI 1	0.020	12.25
PGM 1	0.023	10.62
EST 1	0.021	11.65
MDH 2	0.032	7.56
IDH 1	0.019	12.91
GOT 2	0.031	7.81
α GPD 1	0.029	8.37
SOD 1	0.145	1.47
LDH 1	0.012	20.58
MDH 1	0.141	1.52
GAM 1	0.063	3.71
HEX 1	0.034	7.10
GOT 1	0.033	7.33
FUM 2	0.129	1.68
Mean	0.048	4.96

b)

$P(1)$	Nm^*	Average sample size	Number of populations
0.0607	2.62	31.9	5
0.0540	3.17	30.8	4
0.0483	4.11	31.9	3
0.0484	4.46	34.9	2

Table 4. Results of 5 weeks of drift fence trapping within and at the interface of the two habitats: riparian woodland and evergreen woodland

	Trap catches			
	Within-habitat		Between habitats	
	Number	%	Number	%
Riparian	29	16.7	1	3.3
Evergreen	11	57.9	8	42.1

ty prey while they should exclude low-profitability prey from the diet altogether (Riechert 1991). Instead, they exhibited a low attack rate across all prey types (Table 1).

Tests for gene flow among local populations

Electrophoretic analyses. Table 3 provides a summary of the electrophoretic estimates of population mixing for the five samples of *Agelenopsis aperta* collected in the Portal, Arizona area. F_{ST} is the correlation of two randomly chosen alleles in a subpopulation relative to alleles in the whole population and hence has been used in estimating *Nm* where *N* is the effective population size and *m* is the effective proportion of immigrants (Wright 1931). Wright (1931) determined that $Nm > 1$ (0.5 for diploid organisms) is sufficient to overcome the effects of genetic drift and that $Nm > 4$ indicates that there has been general mixing of the local populations. Table 3a shows that all of the *Nm* values for the polymorphic loci scored are above 1 and most are above 4, suggesting that all *A. aperta* in the area belong to one panmictic population.

Most important to this study is the level of genetic mixing that occurs between spiders in the riparian woodland habitat and adjoining habitats. Slatkin (1985b) suggests that the private allele estimate of *Nm* can be used to test for spatial relationships in gene flow since computer simulations have shown this measure to be relatively insensitive to most parameters other than *Nm* and the number of individuals sampled per population (for which a correction can be applied). The results of the private allele estimate of *Nm* are presented in Table 3b. As expected for an animal that disperses only by ground, *Nm* increases in value with decreasing distance between the riparian woodland and other local populations sampled in the area. The private allele estimates of *Nm* for riparian woodland and its two closest neighbors (intermediate riparian and the adjacent evergreen woodland) are both in excess of 4, indicating that there is general genetic mixing between each of these habitats and the riparian woodland area (Wright 1931).

Spider movement. Results of the drift-fence collections suggest that there is largely unidirectional movement of evergreen woodland spiders into the riparian woodland habitat (Table 4). Of the total evergreen-woodland spider movement 42% was into the riparian woodland habitat (individuals collected in interface traps) while only 3% of the total movement of riparian-woodland spiders was into the evergreen woodland habitat. The evergreen-woodland spiders exhibited significantly more cross-habitat movement than did the riparian woodland animals (*G*-test, $G=11.4$, $P<0.001$). Adult male *A. aperta* were the main movers (55.6%), not surprising given the fact that data collection coincided with male movement in search of matings. Adult females made up 27.1% of the trap captures and immatures the remaining 17.4%.

Experimental test for gene flow effects

Fewer *A. aperta* remained in the exposed enclosures after 6 months of selection than in the protected enclosures (mean ± 95% confidence interval, CI: exposed=10.8, 6.3–15.2; protected=19.0, 14.5–23.5). A one-way analy-

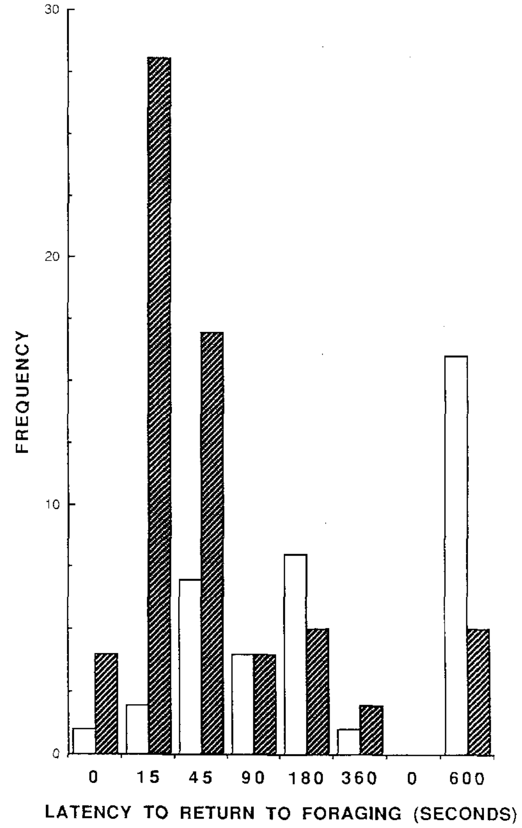

Fig. 3. Latencies to return to a foraging mode at the funnel entrance of the web following the presentation of a predator cue for individuals collected from enclosures that either were exposed to bird predation (*blank columns*) or were protected from it (*solid columns*) for 6 months. Data summaries from four replicates of each treatment. (Note that all enclosures had been initially innoculated with the same familial representation of spiders)

Table 5. Comparisons of predator cue test results for offspring of selected spiders collected from one replicate enclosure of exposed treatment with those from each of 4 replicate protected treatment enclosures (t-tests, $n=15$ individuals/class)

Class	Latency to return to foraging (s)		
	Mean	SE	*P*
Exposed 1	1215.9	27.1	
vs			
Protected 1	407.2	26.0	0.0006
Protected 2	449.3	54.2	0.015
Protected 3	161.3	21.6	0.00002
Protected 4	316.6	36.3	0.0006

sis of variance detected a significant between treatment difference in spider numbers ($P=0.019$).

Further, a one-way analysis of variance applied to latency of the return to foraging following the presentation of a predator cue indicates that spiders sampled from the exposed enclosures were significantly more "fearful" (had longer latencies for return to foraging) than spiders sampled from the protected enclosures ($P<0.0008$: Fig. 3). The majority of the contest trials between equal-weight exposed-treatment versus protected-treatment spiders were won by protected-treatment spiders (74%, $n=20$).

Similar results were obtained for the offspring of the spiders subjected to different predation pressures. No spiderlings were found in three of the exposed enclosures after selection, indicating that birds had eliminated spiders from these plots. All four of the protected enclosures had spiderling *A. aperta*. The results of the predator cue test on offspring of the selected spiders are presented in Table 5. Because only one of the exposed enclosures produced individuals, I used a *t*-test to compare the fear scores from the single replicate of the exposed treatment to those of each of the four protected-treatment replicates. The offspring of spiders exposed to bird predation exhibited significantly longer latencies for return to foraging following a predator cue than did any of the four replicate groups of spiders associated with the protected treatment. The results of the contest trials again were similar to those obtained for the parent generation. Of the 14 contests involving individuals matched for weight, 12 were won by individuals from the protected treatment.

Discussion

Selection pressures and spider behavior

With the exception of the riparian woodland, all of the habitats occupied by *A. aperta* in the Portal, Arizona area afford this spider only limited food and web-sites. These conclusions are evident from the prey availability and behavioral data. Prey availabilities in these habitats are more similar to the aridland NM habitat included for comparison than they are to the riparian woodland. Further, competition for web-sites appears to be high in the more arid Arizona habitats as evidenced by the high frequency of owner choice of escalation as a strategy in the contests I enduced. Finally, high attack rates are exhibited towards both low- and high-profitability prey types, indicating that spiders in these arid habitats must maintain a broad diet and attempt capture of most prey encountering the web to survive.

Note that the riparian woodland population of *A. aperta* has far greater prey availability levels than any other of the populations in the area (Fig. 2). Yet its behavior, though significantly different from the behavior of the other local populations, nevertheless is more like the behavior of individuals occupying the more arid habitats than expected (See Introduction; Hammerstein and Riechert 1988; Riechert and Hedrick 1990; Riechert 1991).

Gene flow among local populations

Both F_{ST} and private allele estimates of *Nm* obtained from electrophoretic analyses of samples from various habitats in the vicinity of Portal, Arizona indicate that significant levels of gene flow have occurred between riparian woodland *A. aperta* and *A. aperta* occupying more arid habitats in the surrounding area. Drift fence collections indicate that local population exchange regularly occurs but that this exchange is largely unidirectional: I found that spiders moved differentially from the more arid evergreen woodland into the more mesic riparian woodland. These results suggest that individuals occupying the riparian woodland habitat may be a mixture of aridland and riparian phenotypes.

Experimental test for gene flow effects

There is some controversy as to the extent to which gene flow may overcome strong selection (reviewed in Slatkin 1985a). The results of my exclusion experiment in the AZ riparian woodland habitat clearly indicate that one generation of predation pressure in the absence of gene flow is sufficient to produce a marked shift in behavior towards more fearful spiders. Because the offspring produced by individuals that had been subjected to different selection pressures scored similarly to the parent generation for the respective treatments, I conclude that this shift reflects selection against the more aridland phenotypes in the exposed enclosures.

This experiment also demonstrates that emerging riparian woodland spiderlings are naturally a mixture of aridlands (aggressive) and woodland (fearful) phenotypes. This finding offers strong support of the view that gene flow can limit adaptation to local conditions. These results conflict with the prevailing view of gene flow as being insufficient to counter strong selection pressure (Brandon 1990). Just as depicted of the Red Queen in *Alice in Wonderland*, the Az riparian-woodland *A. aperta* population seems to be running as fast as it can just to stay in place, but for a different reason from that in van Valen's (1973) analogy where the problem is one of maintaining adaptation in a changing environment.

Unidirectional dispersal

Reduced dispersal (Brandon 1990) and flexible behavior (Sultan 1987) are solutions postulated for systems in which the genetic neighborhood is larger than the size of the selective environment. *Agelenopsis* exhibits neither of these solutions. The fact that gene flow is largely unidirectional may explain *A. aperta*'s failure to have arrived at these solutions.

Via (1991) also failed to detect flexibility in the response of pea aphids to a spatial environment offering two host plants, though Moran (1991) reports alternative developmental pathways as a solution in another aphid system. Despite moderate levels of migration between patch types, Via did not find clones of aphids that performed equally well on both crops. She concludes that there is some genetic constraint to flexible responses. She also suggests that dispersal in this case might not be equated with gene flow, in that intercrop movement does result in significantly reduced longevity and fecundity for the individuals that exhibit it. While the potential for gene flow is there, the selective disadvantage may limit reproduction of dispersers. The extent to which dispersal equates with gene flow is a question I am currently working on. I expect that gene flow levels are high, however, since my indirect test for it (electrophoretic analyses) indicates that the riparian woodland population and its near neighbors are panmictic. As Via and Lande (1985) concluded, even a small amount of migration should eventually homogenize subpopulations genetically.

Further study of the interaction between gene flow and predation is needed in the AZ riparian woodland system to establish the importance of this mixing phenomenon. It does appear that the selectively homogeneous environment for *A. aperta* is smaller than its genetic neighborhood. This mixing of phenotypes that have been selected for in different environments causes significant deviation of riparian woodland spiders from predicted equilibrium behavior.

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References

Brandon RN (1990) Adaptation and environment. Princeton University Press, Princeton

Brooks DR, Wiley EO (1988) Evolution as entropy: toward a unified theory of biology. University of Chicago Press, Chicago

Cheverud JM, Dow MM, Leutenberger W (1985) The quantitative assessment of phylogenetic constraints in comparative analyses. Sexual dimorphism in body weight mong primates. Evolution 39:1335–1351

Grafen A (1991) Modelling in behavioral ecology. In: Krebs JR, Davies NB (eds) Behavioural ecology. Blackwell, Oxford

Hammerstein P, Riechert SE (1988) Payoffs and strategies in spider territorial contests: ESS-analyses of two ecotypes. Evol Ecol 2:115–138

Harris H, Hopkinson HA (1978) Handbook of enzyme electrophoresis in human genetics. Elsevier, New York

Hedrick AV, Riechert SE (1989) Population variation in the foraging behavior of a spider: the role of genetics. Ecologia 80:533–539

Krebs JR, Davies NB (1981) An introduction to behavioural ecology. Sinauer, Sunderland Mass

Lewontin RC (1987) The shape of optimality. In: Dupee J (ed) The latest on the best: essays on evolution and optimality. MIT Press, Cambridge Mass

Lowe CH (1964) The vertebrates of Arizona. University of Arizona Press, Tucson

Maynard Smith J (1978) Optimization theory in evolution. Annu Rev Ecol Syst 9:13–56

Maynard Smith J, Riechert SE (1984) A conflicting tendency model of spider agonistic behaviour: hybrid-pure population line comparisons. Anim Behav 32:564–578

McCracken GF, Wilkinson GS (1988) Allozyme techniques and kinship assessment in bats. In: Kunz TH (ed) Behavioral and ecological techniques for research on bats. Smithsonian Press, Washington DC

Moran NA (1991) Phenotype fixation and genotypic diversity in the complex life cycle of the aphid *Pemphigus getae*. Evolution 45:957–970

Pyke GH (1984) Optimal foraging theory: a critical review. Annu Rev Ecol Syst 15:523–575

Reynolds JB, Weir S, Cockerham CC (1983) Estimation of the coan-cestry coefficient: basis for a short-term genetic distance. Genetics 105:767–779

Riechert SE (1978) Energy-based territoriality in populations of the desert spider *Agelenopsis aperta* (Gertsch). Symp Zool Soc London 42:211–222

Riechert SE (1981) The consequences of being territorial: spiders, a case study. Am Nat 117:871–892

Riechert SE (1991) Prey abundance versus diet breadth in a spider desert test system. Evol Ecol 5:327–338

Riechert SE (1993a) The evolution of behavioral phenotypes: lessons learned from divergent spider populations. Adv Anim Behav 22:130–134

Riechert SE (1993b) A test for phylogenetic constraints on behavioral adaptation in a spider system. Behav Ecol Sociobiol 32:343–348

Riechert SE, Hedrick AV (1990) Levels of predation and genetically based anti-predatory behavior in the spider, *Agelenopsis aperta*. Anim Behav 40:679–687

Riechert SE, Maynard Smith J (1989) Genetic analyses of two behavioural traits linked to individual fitness in the desert spider, *Agelenopsis aperta*. Anim Behav 37:624–637

Selander RK, Smith MH, Yand SY, Johnson WE, Johnson JB (1971) Biochemical polymorphisms and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse. Stud Genet Univ Tex Publ 7103:49–90

Slatkin M (1985a) Gene flow in natural populations. Annu Rev Ecol Syst 16:393–430

Slatkin M (1985b) Rare alleles as indicators of gene flow. Evolution 39:53–65

Slatkin M, Arter HE (1991) Spatial autocorrelation methods in population genetics. Am Nat 138:499–517

Sokal RR, Oden NL (1978) Spatial autocorrelation in biology. II. Some biological implications and four applications of evolutionary and ecological interest. Biol J Linn Soc 10:229–249

Sultan SE (1987) Evolutionary implications of phenotypic plasticity in plants. In: Hecht MK, Wallace B, Prance GT (eds) Evolutionary biology. Plenum Press, New York

Swofford DL, Selander RB (1981) Biosys-1: A computer program for the analysis of allelic variation in genetics. University of Illinois Press, Urbana

Valen L Van (1973) A new evolutionary law. Evol Theor 1:1–30

Valen L Van (1976) Ecological species, multispecies, and oaks. Taxon 25:233–239

Via S (1991) The genetic structure of host plant adaptation in a spatial patchwork: demographic variability among reciprocally transplanted pea aphid clones. Evolution 45:827–852

Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522

Wanntorp H-E, Brooks DR, Nilsson T, Nylin S, Ronquist F, Stearns SC, Wedell N (1990) Phylogenetic approaches in ecology. Oikos 57:119–132

Wright S (1931) Evolution in mendelian populations. Genetics 16:97–159