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Danoff-Burg & Dunn: Beetle road avoidance proximate cues

Why did the beetle not cross the road?:

Proximate stimuli for avoiding road crossings

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ABSTRACT

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2
3 Roads create gaps in forests that pose barriers to many animals. However, little is known about the
4 proximate cues used by animals to detect and avoid these barriers. We studied this phenomenon by
5 gauging the vagility of three species of carrion beetles (*Nicrophorus tomentosus*, *Ni. orbicollis*, and
6 *Necrophila americana*), as measured by walking rate, proclivity for flight, and preference for flying
7 or walking. We demonstrated that even those roads that are minimally repellent such as single-lane
8 dirt roads with low traffic volume were repellent to all three species. We tested the following six
9 proximate stimuli present on roads as repellent forces: dryness, absence of litter, heat, windiness,
10 brightness, and depth of field. All three species avoided some but not all six stimuli that are associated
11 with the road. The beetles responded to the three aerially-sensed stimuli in all possible manners. In
12 contrast, the three terrestrially-sensed stimuli were all either repellent or unimportant to all three
13 species. Litter absence was the most consistently repellent stimulus. Litter absence is the only
14 temporally invariant stimulus and is therefore the most reliable cue that the beetles could use to avoid
15 roads. Our results support the hypotheses that even minimal impact single-lane dirt roads inhibit
16 beetle movement, that the roads are repellent irrespective of beetle species vagility, and that this
17 barrier effect is primarily due to the repellency of a temporally invariant proximate cue. We have
18 therefore linked micro-environmental changes due to forest fragmentation by roads to specific beetle
19 behaviours.

20
21 (Word count for abstract: 245)

1 The network of roads in the U.S. and worldwide continues to increase and dissect landscapes into
2 smaller and smaller fragments. For example, in Germany roads constitute 3.6 km / km² of the total
3 land area (Mader et al. 1990). Dissected landscapes are further divided by finer scale linear
4 disturbances such as dirt roads and footpaths. These roads affect forests by further reducing the total
5 undisturbed area, increasing edge effects, diminishing habitat quality, providing corridors for invasive
6 species, and creating barriers to animal movement (Burnett 1992).

7
8 Roads have been demonstrated to act as barriers to the movement of many animals. Species
9 of carabid beetles (Mader et al. 1990; Mader 1984), snails (Baur & Baur 1990), birds and mammals
10 (Goosem 1997) have all been shown to avoid crossing roads. The barrier effect of these roads could
11 lead to a selection against less vagile individuals and species (Thomas et al. 1998). At its most
12 extreme, the barrier effect of roads can completely isolate populations of species with very low
13 vagility. Fragmentation of populations can reduce genetic variability and increase population
14 extinction rates (Nason et al. 1997), which can lead to loss of local community diversity (Didham et
15 al. 1998), and possibly decreased ecosystem functioning (Naeem et al. 1994; Didham et al. 1996). To
16 minimize the effects of fragmentation by roads, we must first understand how animals sense barrier
17 effects.

18
19 The proximate cause of the road-based barrier effect is little understood. Roads may prevent
20 animal crossing due to direct road-related mortality (Fahrig et al. 1995). It is also possible that the
21 animals can cross roads but once across, they cannot invade established territories of conspecifics
22 (Oxley et al. 1974). However, in studies where the effects of direct mortality and the inability to
23 invade conspecific territories across roads were eliminated (by using two-lane dirt roads and then
24 looking at the initial choice of direction), animals still preferred to not cross roads (Mader et al.
25 1990). Environmental stimuli associated with roads may then be an important part of the barrier
26 effect.

27

1 To an animal at a roadside, the differences between the stimuli associated with the road
2 environment and those of the undisturbed surrounding environment are potentially great. The
3 proximate stimuli associated with roadsides have been suggested to be sufficiently strong to prevent
4 some animals from even approaching within hundreds of meters of a road (Goosem 1997).

5
6 We sought to explore whether and how a minimally invasive road type affected animal
7 movement. In this paper we had three goals. First we evaluated the vagilities of three species of
8 burying beetles, as measured by walking rate, proclivity for flight, and their preference to fly or walk.
9 Second, we tested the hypothesis that infrequently used 3 m wide dirt roads hinder beetle movement.
10 Last, we individually examined six proximate stimuli that the beetles could have used in deciding
11 whether or not to cross these roads. We tested the hypotheses that each of these road-associated
12 proximate stimuli discourage road crossings and that the response patterns of the beetles are
13 determined by the most environmentally reliable stimuli.

METHODS

Beetles used

Three species of beetles in the family Silphidae were chosen for this experiment based on local abundance and to represent a variety of levels of vagility within a trophic guild. All three species are large, forest litter-dwelling, carrion feeders capable of strong flight (Anderson & Peck 1985). These species compete for the same resources locally (Anderson 1982; Wilson et al. 1984), play a key role in necromass recycling (Anderson & Peck 1985), and are found throughout eastern North America (Peck & Kaulbars 1987). *Necrophila americana* and *Nicrophorus tomentosus* are primarily diurnally active species, whereas *Nicrophorus orbicollis* is primarily nocturnal (Wilson et al. 1984; Anderson & Peck 1985). Adults of all three species live for two to four months (Anderson & Peck 1985) and are capable of rapid short-distance walking as well as longer distance flight.

All beetles were collected from baited pitfall traps placed around the Black Rock Forest (BRF) in Cornwall, New York. This forest is a predominantly oak-dominated, mixed hardwood, deciduous forest, located 41° 24' N by 74° 2' W. Collection sites were located at elevations between 305 and 400 m above sea level. All animals were collected and all trials were conducted between 6-14 August 1998 at the BRF.

Vagility trials

Two vagility tests were conducted to examine the relative proclivity to flight and walking speed of the three silphid species. These tests allowed us to discern which species are more likely to walk or fly. A species that was more likely to fly was considered to be more vagile than one more likely to walk.

1 In the first test, the "choice test", the beetles were placed in an indoor circular experimental
2 arena with a 2.2 m radius and were allowed to escape by walking or flying. A series of six smaller rings
3 were drawn concentrically at 31 cm intervals using chalk. Four lights were placed equidistant from
4 each other around the ring perimeter. Although we strove for uniform lighting, weak indirect light
5 also came from one direction through an open door that was 15 feet away. Beetles were released in
6 the centre of the ring and the displacement mode, time to completely escape arena, and movement
7 direction were recorded. Individuals that engaged in circular local search were excluded (2 *Ne.*
8 *americana* and 1 *Ni. orbicollis* individuals). Forty replicates were conducted for each beetle species
9 and each replicate used a unique individual.

10
11 The second vagility test, the "forced flight test", constrained the beetles to escape by flight.
12 A single beetle was placed in the centre of each uncovered, opaque plastic bucket (20 cm diameter x
13 15 cm height). Whether each beetle took flight and how long it took to fly was recorded. A
14 maximum of ten minutes was allowed for each replicate, after which time non-flying beetles were
15 tallied and then released. Sixty replicates were conducted for each species, including non-flying
16 individuals, and unique individuals were used for each replicate.

17
18 Data from these vagility tests were analyzed in three manners. First, Chi-square tests were
19 performed comparing the number of individuals of each beetle species that took flight within each
20 test to each of the other two species. Second, an ANOVA was performed to determine whether the
21 time it took to walk out of the arena in the choice test significantly differed between species. Last,
22 an ANOVA was performed comparing the time to flight for each species in the forced flight test only
23 including those individuals that flew within ten minutes.

24
25 Road crossing

26

1 To evaluate whether roads affected these three silphids, we conducted roadside release
2 experiments in the BRF. We tested the hypothesis that beetles will be less likely to cross minimal
3 impact unpaved single lane roads than to go directly into the forest. A species was said to generally
4 avoid crossing roads if a significantly greater number of individuals went into the forest rather than
5 across the road. We hypothesized that those species that were more likely to fly (more vagile) would
6 be less affected by the presence of roads than would those that are more pedestrian.

7
8 All road crossing experiments were conducted aside dirt roads that were approximately 410 m
9 above sea level. Four sites were selected within the BRF on sections of single-lane roads bordered on
10 both sides (within 0.1 m of edge of road) by forest with a dense undercanopy. Less than ten vehicles
11 drove on the roads per day and all at low speeds (20 km/h or less). Trial temperatures ranged between
12 26-31 °C and winds were still throughout the trials. Trials were conducted between 1100 and 1600
13 hours. Sites were selected to have no slope on either the road surface or on their verges, to receive
14 direct unfiltered sunlight at mid-day, to have only sand on the road surface, and to have unique
15 compass orientations. Road site 1 was oriented N-S, site 2 NW-SE, site 3 NNE-SSW, and site 4 E-W.
16 Each replicate consisted of a beetle being released at the edge of the road at the border between the
17 shaded leaf litter and the sunny dirt road. The initial direction the beetle moved from the release site,
18 its displacement mode, displacement path, and final location after 30 seconds were recorded. At each
19 site 30 replicates were conducted for each beetle species on both sides of the road and unique
20 individuals were used for each of the sixty replicates per site. *Ni. tomentosus* was not tested at site 4
21 because an inadequate number of these beetles were captured.

22
23 Three sets of Chi-square tests were performed on the road test data, with the quantities that
24 crossed the road versus those that did not as the dependent variable. First, significant differences in
25 beetle behaviour within each species between road sides were tested. Data from alternate sides of a
26 road within a site that did not differ were subsequently pooled. Second, significant differences within
27 each species at the four sites were tested. Sites that did not significantly differ within a species were

1 then combined for subsequent analyses. Third, whether each species significantly avoided crossing
2 roads was tested using data pooled from both road sides and from all four sites.

3

4 Proximate stimuli

5

6 Using laboratory-based choice tests, six stimuli that generally differ between road and forest
7 environments during midsummer middays were tested for their importance as proximate cues for
8 roadside avoidance. The six stimuli were the presence or absence of moisture, leaf-litter, heat, wind,
9 light, and depth of field. We considered roads to be drier, with less leaf-litter, hotter, windier,
10 brighter, and with a greater depth of field than the surrounding forest. A stimulus was considered to be
11 used for road avoidance if the form of the stimulus characteristic of roads was avoided. We
12 hypothesized that species that are more likely to walk than fly should be most sensitive to those
13 stimuli that are strongly apparent terrestrially such as dryness, leaf-litter, temperature of the sand,
14 and brightness. We also hypothesized that species that are more likely to fly should be most sensitive
15 to stimuli best sensed aerially such as windiness, brightness, and depth of field.

16

17 All three silphid species were individually tested for all six stimuli. Choice tests were
18 conducted in cardboard box arenas measuring 45 cm w by 49 cm h by 60 cm l. The arena bottom was
19 filled with 5 cm of sterile sand and operationally divided along its length into two equal parts. A
20 single 60w incandescent light bulb lit the arena and hung 2m above the centre of the arena.

21

22 During the six sets of proximate stimulus tests, all variables except the one being studied were
23 kept uniform throughout the arena. For moisture trials, one half of each box was uniformly sprinkled
24 with 200 ml water. A strip of plastic was placed under the surface of the sand along the centre
25 dividing line to prevent the spread of water from the wet to the dry side. During the litter trials, half
26 of the arena bottom was covered 8 cm deep with sifted leaf-litter. All leaf-litter was sterile and sun-
27 dried to remove excess moisture. For the heat trials, a hot plate was put under one side of the box and

1 covered with a metal plate equal to half of the arena which uniformly heated the sand on that half to
2 55°C, whereas the cool side remained at 35°C.

3
4 The experimental arena was further modified for the proximate stimulus tests of the final
5 three stimuli. A cardboard barrier that spanned the width of the arena along the central dividing line
6 was added to the arena for the wind and light choice tests. The cardboard began 3 cm above the sand,
7 continued to the top of the arena, and served as a barrier to wind and light. During the wind trials, the
8 entire top of the arena was open, a separate 60 w incandescent bulb was suspended over the centre of
9 both sides of the arena, and a light breeze was supplied over one side of the box using a small fan. A
10 hole was cut across the entire arena side perpendicular to the centre dividing line to allow the wind to
11 exit the arena. This hole was covered with fiberglass window screening and was hooded to keep
12 outside light from entering the box through this gap. In the light trials, a piece of cardboard was
13 placed over one half of each box and a clear shadow edge was formed along the centre dividing line.
14 For the depth of field tests, the central cardboard barrier and the wall at one end of the arena (parallel
15 to the central dividing line) were removed.

16
17 A replicate was begun by placing a single beetle in the centre of the arena and the initial
18 movement direction (relative to the two experimental choices), displacement mode, and final
19 location were recorded for each beetle replicate. The final location was when the beetle reached an
20 end of the arena. Sixty replicates were conducted for each stimulus for each species. Half of the
21 replicates for each stimulus were conducted with the arena in one orientation and the other half were
22 conducted with the arena rotated 180° to control for room effects. For those stimuli where a barrier
23 along the centre of the arena was used (light and wind), half of the beetles were introduced from each
24 side of the barrier, alternating introduction sides every five replicates. A unique individual was used
25 for each replicate for each stimulus.

26

1 Two Chi-square tests were performed for each species using the proximate stimulus choice
2 test data, with the quantities of beetles that chose each experimental condition as dependent
3 variables. First, a Chi-square test was performed to test for significant differences in beetle behaviour
4 between arena orientations due to room effects. Data that did not differ between arena orientations
5 were then pooled for the subsequent analysis. Second, a Chi-square test was performed to test whether
6 each of the six stimuli significantly affected beetle movement.

RESULTS

Vagility tests

Of the three silphid species, *Ne. americana* was the species most likely to escape by walking. It was the least likely to fly when given the opportunity to choose between escaping by walking or by flying (choice vagility test; Chi-square test: $\chi^2_2=8.2$, $P<0.05$; Fig. 1), did not fly when given the choice to do so (choice vagility test, Fig. 1), and was the slowest to flight when forced to escape by flying (forced flight vagility test; ANOVA: $F_{2,117}=7.4$, $P<0.001$; Fig. 2). When forced to escape by flight (forced flight vagility test), only half of the *Ne. americana* individuals chose to fly within the ten-minute experimental period (Fig. 1).

In contrast to the other species, *Ni. tomentosus* was significantly more likely to fly when given the opportunity to choose (Chi-square test: $\chi^2_2=38.0$, $P<0.001$; Fig. 1) and covered the greatest total number of sectors in flight (Chi-square test: $\chi^2_2=197.2$, $P<0.001$; Fig. 1). *Ni. tomentosus* did so the quickest of the three species (when compared to *Ni. orbicollis*, Paired t-test: $t_{103}=2.4$, $P<0.01$; when compared to *Ne. americana*, Paired t-test: $t_{82}=4.3$, $P<0.001$), and all species took to flight at different speeds when forced to escape by flying (ANOVA: $F_{2,132}=7.5$, $P<0.001$; Fig. 2). Although it did not exit the arena the quickest (Fig. 2), the mean time to exit the choice test arena was not reflective of the activity level of *Ni. tomentosus*. These beetles anecdotally walked the quickest of the three, but would frequently pause for many seconds before resuming movement. In short, this species was capable of the most rapid walking, but in bursts, and was also the most likely of the three species to fly.

Nicrophorus orbicollis was intermediate between the other two species in how often they relied on flight for escape. When it was given the choice for mode of escape, *Ni. orbicollis* overwhelmingly chose to walk (Fig. 1). The three individuals that did take flight in the choice test

1 first walked through at least half of the arena before flight. Of the three species, *Ni. orbicollis* walked
 2 the fastest (ANOVA: $F_{2,117}=7.4$, $P<0.001$; Fig. 2), and did not pause before exiting the arena.
 3 Although *Ni. orbicollis* was not significantly different from *Ne. americana* in the number of
 4 individuals that flew in the choice test, it took flight significantly sooner than did *Ne. americana*
 5 when forced to escape by flying (Fig. 2).

7 Road crossing

9 No statistically significant differences in beetle crossing frequencies were obtained from Chi-
 10 square tests either when roadsides within a site (*Ne. americana* Chi-square test: $\chi^2_7=12.8$, NS; *Ni.*
 11 *orbicollis* Chi-square test: $\chi^2_7=2.1$, NS; *Ni. tomentosus* Chi-square test: $\chi^2_7=2.0$, NS) or when all sites
 12 (with the two roadsides pooled) were compared within any of the three silphid species (*Ne.*
 13 *americana* Chi-square test: $\chi^2_{15}=17.2$, NS; *Ni. orbicollis* Chi-square test: $\chi^2_{15}=10.0$, NS; *Ni.*
 14 *tomentosus* Chi-square test: $\chi^2_{15}=11.0$, NS). All data were then pooled within species, irrespective of
 15 road side or road site. Pooling provided a total of 240 replicates for *Ne. americana* and *Ni. orbicollis*
 16 and 180 replicates for *Ni. tomentosus*.

18 All three species significantly avoided crossing the road (*Ne. americana* Chi-square test:
 19 $\chi^2_1=104.0$, $P<0.001$; *Ni. orbicollis* Chi-square test: $\chi^2_1=93.8$, $P<0.001$; *Ni. tomentosus* Chi-square
 20 test: $\chi^2_1=10.7$, $P<0.01$). The two species that were least likely to fly in the choice vagility tests were
 21 the most affected by the road (82.9% of *Ne. americana* and 81.3% *Ni. orbicollis* did not cross),
 22 whereas a smaller percentage of the species most prone to flight (62.2% of *Ni. tomentosus*) did not
 23 cross the road (Fig. 3). *Necrophila americana* and *Ni. orbicollis* were similar to each other in nearly
 24 all their responses to roadside releases (Fig. 3, Table 1).

26 The beetle species differed in their mode of movement away from the release site (Table 1).
 27 The most pedestrian silphid species in the vagility tests (*Ne. americana*) flew only 16.2% of time

1 from the release site (8.3% of all replicates into the forest and 7.9% across the road). The species
2 that was the most prone to flight (*Ni. tomentosus*) flew from the roadside release sites the greatest
3 percent of time (56.1% of all replicates, 22.2% into the forest and 33.9% across the road).

4 *Nicrophorus orbicollis* was intermediate between these two species , but at rates most similar to *Ne.*
5 *americana*, and took flight 24.6% of the time (7.1% into the forest and 17.5% across the road).

6
7 The beetles overwhelmingly preferred to enter the forest by walking (Table 1). On average,
8 only 15% of moves into the forest were by flight for all species (*Ne. americana* 10.5%, *Ni. orbicollis*
9 8.7%, and *Ni. tomentosus* 35.7%). However, at least half of the movements across the road were by
10 flying. Whereas the most pedestrian of the three species (*Ne. americana*) flew across the road as
11 often as it walked across (46.3%), both *Nicrophorus* species mostly flew across the road (93.3% of
12 *Ni. orbicollis* flew and 89.7% of *Ni. tomentosus* flew).

13
14 The two species least likely to fly in the vagility tests were most likely to walk rather than
15 fly when their initial movement was along the road (66.7% *Ne. americana* walked and 88.9% *Ni.*
16 *orbicollis* walked). In contrast, *Ni. tomentosus* was much less likely to walk than fly when initially
17 moving along the road (18.2% walked). The *Nicrophorus* species were similar in the percentage of all
18 flights that were initially parallel to the roads. A small percentage of *Ni. orbicollis* (5.1%) and *Ni.*
19 *tomentosus* (17.8%) flights were initially parallel to the road (Table 1), whereas 35.9% of *Ne.*
20 *americana* initially flew parallel to the road. Eventually, all individuals that initially flew parallel to
21 the road entered the forest in flight, either with or without crossing the road.

22
23 Proximate stimuli

24
25 Room effects due to arena orientations were not significant for all six stimuli within all three
26 species (all Chi-square tests: $\chi^2_1 < 3.7$, NS). All replicates were pooled within a species for each
27 stimulus during subsequent analyses.

1
2 In general, no two silphid species responded identically to the six proximate stimuli, but
3 nearly all of their choices were consistent with avoiding roads (Table 2). The congeneric *Nicrophorus*
4 species were not more similar in their responses to the proximate stimuli than either was when
5 compared to *Ne. americana*. *Nicrophorus tomentosus* and *Ni. orbicollis* differed in their response to
6 two stimuli, *Ni. tomentosus* and *Ne. americana* differed in two stimuli, and *Ni. orbicollis* and *Ne.*
7 *americana* responses differed in four stimuli (Table 2).
8

9 The silphids did not often change their preferences during the proximate cue tests. Overall,
10 only 12.1% of any beetles in any of the proximate cue tests changed their final choice relative to
11 their initial choice during an experimental replicate (15.5% of *Ne. americana*, 9.4% of *Ni. orbicollis*,
12 and 11.4% of *Ni. tomentosus*). Only one species significantly changed its final preference relative to
13 its initial preference, and then concerning a single presented stimulus. The presence or absence of
14 litter did not significantly determine the initial direction of *Ni. orbicollis* (Chi-square test: $\chi^2_1=1.1$,
15 NS), but litter presence was significantly preferred at the end of the trial (Chi-square test: $\chi^2_1=5.4$,
16 $P<0.05$; Table 2). Because of the trivial differences observed between beetle behaviour at the
17 beginning and end of the experimental replicates, we will continue to discuss only the final results.
18

19 The three silphids responded identically to only two proximal stimuli (Table 2). The
20 presence or absence of moisture was not significantly used as a stimulus for choosing movement
21 direction in any species (*Ne. americana* Chi-square test: $\chi^2_1=0.3$, NS; *Ni. orbicollis* Chi-square test:
22 $\chi^2_1=1.1$, NS; *Ni. tomentosus* Chi-square test: $\chi^2_1=0.1$, NS). However, absence of leaf-litter was
23 significantly repellent to all three species as the basis for their final directional choice (*Ne.*
24 *americana* Chi-square test: $\chi^2_1=28.5$, $P<0.001$; *Ni. orbicollis* Chi-square test: $\chi^2_1=5.4$, $P<0.05$; *Ni.*
25 *tomentosus* Chi-square test: $\chi^2_1=24.1$, $P<0.001$).
26

1 Heat and wind were repellent to only a single species each (*Ni. orbicollis* heat Chi-square test:
2 $\chi^2_1=15.0$, $P<0.001$ and *Ne. americana* wind Chi-square test: $\chi^2_1=17.1$, $P<0.001$; Table 2). The other
3 two species did not make a significant choice during tests of either heat (*Ne. americana* Chi-square
4 test: $\chi^2_1=0.6$, NS; *Ni. tomentosus* Chi-square test: $\chi^2_1=0.6$, NS) or wind (*Ni. orbicollis* Chi-square test:
5 $\chi^2_1=2.4$, NS; *Ni. tomentosus* Chi-square test: $\chi^2_1=3.3$, NS).

6
7 Given that all three species significantly avoided crossing roads during roadside releases, the
8 last two stimuli gave conflicting results. Light was significantly repellent for both *Nicrophorus*
9 species (*Ni. orbicollis* Chi-square test: $\chi^2_1=13.1$, $P<0.001$; *Ni. tomentosus* Chi-square test: $\chi^2_1=17.1$,
10 $P<0.001$), but light was a significant attractant for *Ne. americana* (Chi-square test: $\chi^2_1=26.7$,
11 $P<0.001$; Table 2). Similarly, although *Ni. orbicollis* did not use depth of field for choosing a
12 direction (Chi-square test: $\chi^2_1=1.7$, NS), both *Ne. americana* and *Ni. tomentosus* were significantly
13 attracted to a greater depth of field (*Ne. americana* Chi-square test: $\chi^2_1=24.1$, $P<0.001$; *Ni.*
14 *tomentosus* Chi-square test: $\chi^2_1=19.3$, $P<0.001$; Table 2).

15
16 In addition to choosing an arena side and moving towards it, flight was also a possible
17 response. Beetles chose to take flight to different degrees in response to different stimuli and did so
18 nearly identically for each stimulus across species. During litter, heat, and moisture proximate
19 stimulus tests, almost no individuals of any species took flight (Table 2). In contrast, during tests of
20 the three stimuli that can be sensed in flight, multiple individuals took flight in all three species (wind
21 [$X \pm SE = 23.3$ individuals per species ± 4.8 , $N = 3$], light [$X \pm SE = 2.3 \pm 0.5$, $N = 3$], and depth of
22 field [$X \pm SE = 6.7 \pm 1.2$, $N = 3$], Table 2).

DISCUSSION

Roads Barriers

Roads served as barriers for all species in this study. All three species, when given the choice whether to cross the road or to move into the forest, chose significantly to move into the forest (Fig. 3).

Others (Mader et al. 1990; Duelli 1990; Goosem 1997) have found that roads are barriers to the movement of a wide taxonomic range of animals. In contrast, Creighton and Schnell (1998) have demonstrated that the American Burying Beetle (*Ni. americanus*) can easily move between adjacent woodland and grassland habitats. Our results indicate not only that these beetles view roads as a categorically different environment from natural habitats, but that even single-lane dirt roads without significant traffic flow or regular road maintenance activities can serve as barriers to beetle movement.

Animals that fly across roads are exposed to the aversive conditions there (higher predation risk, greater exposure, poor habitat, etc.) for less time than are those that walk across. Therefore, those species that move primarily by flight may be less affected by roads. Supporting this expectation, we found that 79.2% of all individuals (averaged across all species) that crossed the road did so by flying across (Table 1). However, we also found that all four of these low-impact dirt roads were significant barriers to all species, irrespective of their vagility (Fig. 3). The species that was the most prone to fly (*Ni. tomentosus*, Fig. 1, Table 1) was only slightly more likely to cross roads than were the two species that primarily walked during the vagility tests (*Ne. americana* and *Ni. orbicollis*, Fig. 1, Table 1). During midsummer middays, these roads prevent the movement of all three species.

Rather than being barriers continually, the beetles may still cross these roads, but at other times of the day and year. We conducted the roadside release tests when the potentially aversive road conditions would be most intense (mid-summer midday). Compared with the field conditions present

1 during our tests, moisture, heat, brightness, and windiness will be less repellent through the day and
2 year. The road barrier effect will be lessened if the cues that the beetles use to avoid these roads are
3 those that change through time.

4
5 Nonetheless, even a less significant or transient barrier effect could present a strong selective
6 force. Less vagile lineages and species have been found to be less present in highly fragmented sites
7 (Thomas et al. 1998; J. A. Danoff-Burg & M. McDonald unpublished data). Prohibitive road
8 conditions are most strongly present diurnally from the late spring to early fall. Because this spans
9 the entire reproductive period for most insects including these beetles, the resulting isolation could be
10 significant.

11
12 Among these three silphid beetles, proclivity to flight (Figs. 1 and 2) tracked road crossing
13 ability (Fig. 3). As such, a partial barrier effect may also select for proclivity to flight among
14 fragmented species. Lineages and species that are more likely to fly would then have an advantage
15 when arriving at ephemeral resources. This hypothesis is supported by data indicating that *Ne.*
16 *americana* (the slowest, least likely to fly, and least likely to cross roads of these three species) is
17 less abundant than *Ni. tomentosus* (the fastest, most likely to fly, and most likely to cross roads of
18 these three species) in areas highly fragmented by roads (J. A. Danoff-Burg & M. McDonald
19 unpublished data). In guilds such as necrotrophs, where competition is high because of limited,
20 patchy, and ephemeral resources, a small temporary decrease in local competitive vigour during
21 reproduction could greatly reduce the long-term fitness of a population (Wilson et al. 1984).

22
23 These road barrier effects could lead to reduced gene flow and a cascade of other effects
24 associated with smaller fragmented populations (Nason et al. 1997), ultimately resulting in local
25 extirpation. A similar process triggered by roads and habitat fragmentation may have caused the
26 American Burying Beetle (*Ni. americanus*) to become an endangered species (Kozol et al. 1988;
27 Ratcliffe & Jameson 1992; Amaral et al. 1997; Creighton & Schnell 1998). Habitat fragmentation by

1 roads could have led to a decrease in both the abundance of the larger small-mammal carcasses
2 (Robinson et al. 1992) that this species exclusively uses to breed and of the appropriate eastern
3 deciduous forest habitat in which it is found (Anderson 1982; Lomolino & Creighton 1996).

4

5 Proximate stimuli

6

7 Although only one proximate stimulus (absence of leaf litter) was universally repellent, four
8 of the six road conditions were repellent for at least one of the three species (Table 2). Moisture was
9 the only stimulus that did not repel at least one species from the road conditions. These results
10 support our initial hypothesis that local environmental stimuli associated with roads are the basis for
11 the beetles not crossing them. This is in contrast to other work suggesting that animals avoid roads
12 due to traffic, roadside chemicals, slope, or territorial conflicts (Mader et al. 1990; Duelli 1990;
13 Goosem 1997).

14

15 Movement Mode

16

17 The beetles made at least two choices in the proximate cue trials. First they chose whether to
18 fly or walk and then they chose the direction of that movement. Based on the data we obtained from
19 the proximate cue tests, we conclude that three primarily diurnal stimuli are likely to be those that
20 trigger flight. Multiple individuals of all three species took flight at the ends of the experimental
21 replicates of the windiness, depth of field, and brightness proximate cue tests (Table 2). Of these
22 three cues, windiness evoked by far the greatest number of flights, indicating that it is the most
23 effective stimulus of these six to trigger flight among these beetles. In the absence of these three
24 cues, the beetles may primarily walk.

25

26 Movement Direction

1
2 Once the beetles chose whether to fly or walk, the direction of movement was their next
3 choice. Ninety percent of the beetles that flew (averaged across all species) crossed the road,
4 suggesting that the stimuli used to decide whether to cross roads differ when flying than when
5 walking. Our data from the proximate cue choice tests (Table 2) are most consistent with the
6 interpretation that terrestrial, road specific stimuli repel walking beetles, whereas aerial stimuli
7 attract flying beetles to the road.

8 9 Terrestrial Stimuli

10
11 When the beetles walked, cues primarily sensed terrestrially (absence of litter, dryness, and
12 heat) either consistently repelled beetles from the road or did not influence their movement
13 direction. Therefore, cues sensed terrestrially are a key component causing the road barrier effect
14 when the beetles are walking. Of these, the most consistently repellent for all species was the absence
15 of litter (Table 2). Litter is always absent from the roads, is always present in the surrounding forest,
16 and does not vary either daily or seasonally in the BRF (J. A. Danoff-Burg unpublished data). Litter is
17 cleared from the road surface by the passage of the few cars that travel on the BRF roads daily.
18 Therefore, litter absence is the most reliable and likely the most important proximate stimulus that
19 these beetles use to avoid roads.

20 21 Aerial Stimuli

22
23 Cues that were best sensed aerially (brightness, depth of field, and windiness) were either
24 inconsistent or did not influence beetle movement when the beetles walked. At least one species was
25 attracted to each of two aerially sensed cues (brightness and depth of field, Table 2). However, wind
26 was repellent for one species and brightness was repellent to two (Table 2). We conclude that when
27 beetles walk, aerial cues are not the basis for road aversion.

1

2 When the beetles are in flight, the movement direction should be determined both by the
3 response patterns of each species (Table 2) and the status of each cue at the encountered roadside.
4 For example, the responses of each species when in flight would differ at a cloudy roadside and all
5 other cues are only weakly apparent. Based on the responses to the aerially sensed proximate cues
6 (Table 2), we would predict that *Ni. tomentosus* should be attracted the road but that *Ni. orbicollis*
7 would either be repelled or not attracted to the road. Depending on the percentage of time these
8 beetles spend in flight, the environmentally dependent responses of these beetles when in flight
9 might make these roads less important barriers.

10

11 Nocturnal activity

12

13 *Nicrophorus orbicollis* behaved somewhat inconsistently with our interpretation in that it was
14 the only species that was not attracted to at least one aerial, road-related stimulus. This nocturnally
15 active species was repelled by brightness but was unaffected by an increase in windiness and the depth
16 of field (Table 2). *Nicrophorus orbicollis* was also the only species to be repelled by heat, a
17 terrestrially sensed road stimulus. The neutrality or aversion of *Ni. orbicollis* to all of these road
18 stimuli may be only an aversion to all diurnal cues. These beetles may cross roads regularly, but only
19 at night. This remains to be tested.

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1
2
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Table 1 - Mode of Road Crossing Data are from the roadside release experiments and are pooled from all four sites. The first two data rows are the numbers of individuals that went into the forest or crossed the road by either walking or by flying. The last row is a subset of the first two and is the number of individuals that moved along the edge of the road prior to either going into the forest or crossing the road.

	<i>Ne. americana</i>		<i>Ni. orbicollis</i>		<i>Ni. tomentosus</i>	
	walking	flying	walking	flying	walking	flying
Went into Forest	179	20	178	17	72	40
Crossed Road	22	19	3	42	7	61
Initially Moved Along Road	30	15	8	1	4	18

Table 2 - Responses to Proximate Stimuli Tests Levels of significance from Chi-square tests of repulsion or attraction to specific cues at the end of the tests (Final) and the number of individuals that flew (# Flew) within each test for each species. Data were pooled from both arena orientations prior to analyses.

Stimulus	<i>Ne. americana</i>		<i>Ni. orbicollis</i>		<i>Ni. tomentosus</i>	
	Final	# Flew	Final	# Flew	Final	# Flew
Dryness (D,N,T)	O	0	O	0	O	0
Absence of Litter (D,N,T)	-***	1	-*	0	-***	1
Ground Heat (D,T)	O	2	-***	0	O	0
Windiness (D,T,A)	-***	21	O	30	O	19
Brightness (D,T,A)	+***	2	-***	3	-***	2
Depth of Field (D,T,A)	+***	5	O	8	+***	7

Abbreviations: "-" cue was repellent, "+" cue was attractant, asterisks denote level of significance "****" $p < 0.001$, "*" $p < 0.05$, "D" cue is present diurnally, "N" cue is present nocturnally, "T" cue is sensed terrestrially when walking, "A" cue is sensed aerially when flying.

FIGURE LEGENDS

Figure 1. Vagility Tests - Numbers of Sectors Flown Across and Individuals Flown. Cross-hatched and black bars are from vagility choice test (Test 1), and the white bars are from the forced flight vagility test (Test 2). Within a category, bars with distinct uppercase bolded letters are significantly different between species at $P < 0.05$. Error bars on the cross-hatched bars signify the standard error and numbers above all bars indicates exact value. Data from Test 1 are based on 40 replicates, data from Test 2 are based on 60 replicates.

Figure 2. Vagility Tests - Median Times to Escape. Cross-hatched bars are from vagility choice test (Test 1) and include only those individuals that walked entirely out of the experimental arena. White bars are from the forced flight vagility test (Test 2) and include only those individuals that took flight in less than 10 min. Within a category, bars with distinct uppercase bolded letters are significantly different between species at or below $P < 0.05$. Error bars signify standard error and numbers above each bar indicates exact value. Data from Test 1 are based on 40 replicates, data from Test 2 are based on 60 replicates.

Figure 3. Road Crossing Tests. Total number of individuals that either crossed or did not cross roads during the roadside release tests. Data are pooled from both road sides and all road sites. Exact bar values are inside bars. All three species significantly preferred not to cross road at $P < 0.001$ (designated with ***).

Figure 1

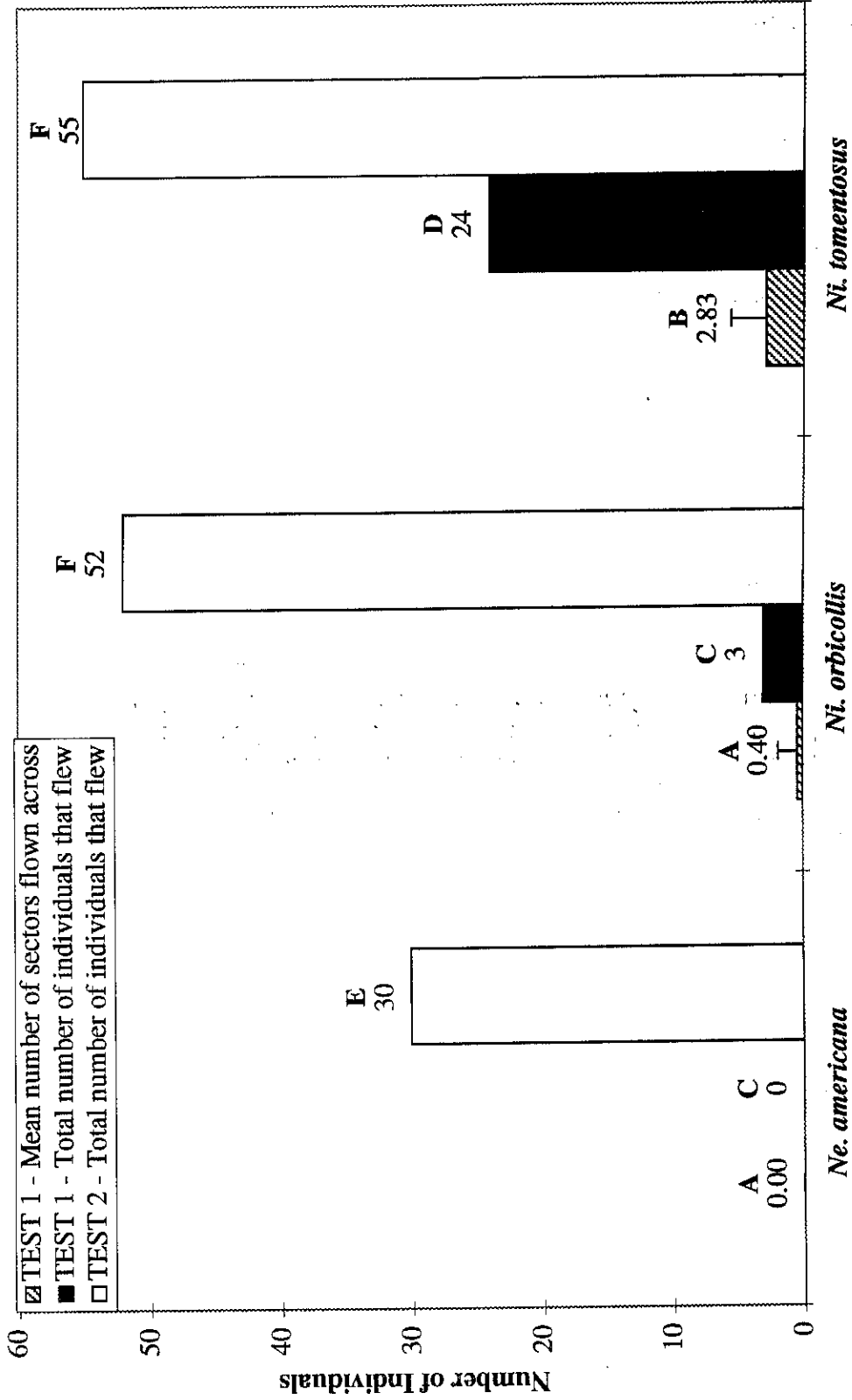


Figure 2

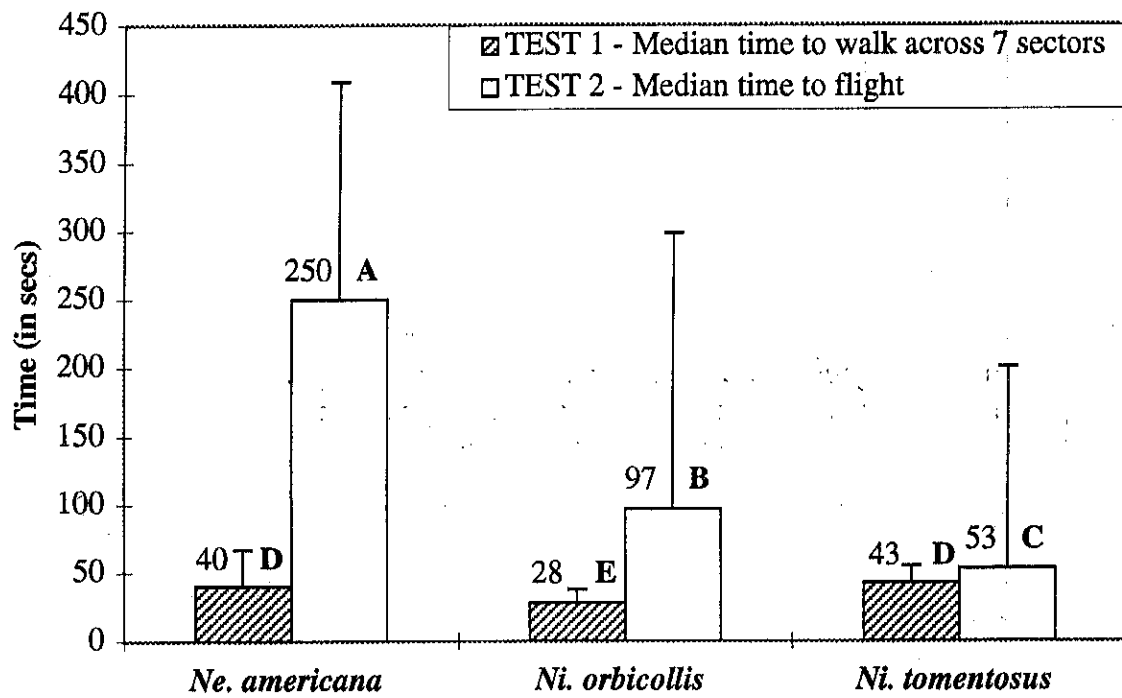


Figure 3

