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FINAL REPORT

SECTION 6

ENDANGERED SPECIES ACT

OKLAHOMA
DEPARTMENT OF WILDLIFE CONSERVATION

FEDERAL AID PROJECT E-13

THE AMERICAN BURYING BEETLE

HABITAT USE AND GENETIC CHARACTERIZATION AND VARIABILITY IN THE AMERICAN BURYING BEETLE, NICROPHORUS AMERICANUS, IN OKLAHOMA

SEPTEMBER 1, 1990 - AUGUST 31, 1994

STATE: Oklahoma

PROJECT NO. E-13-4

PROJECT TYPE: Research

PROJECT TITLE: The American Burying Beetle

SEGMENT DATES: 1 September 1990 - 31 August 1994

STUDY TITLE: Habitat Use and Genetic Characterization and Variability in the American Burying Beetle, Nicrophorus americanus, in Oklahoma.

STUDY OBJECTIVE: To gather data on the habitat use and status of the American burying beetle, Nicrophorus americanus, in Oklahoma and to compare genetic characteristics of the Oklahoma population with that of the New England population.

SUMMARY

We have completed all tasks under the Program Narrative Objectives: I - Field Surveys, II - Habitat Analysis and Interspecific Interactions, III - Captive Rearing of Broods, IV - Collection of Beetles for Genetic Comparisons Between Populations (conducted by A. Kozol), V - Habitat Affinities of Breeding Adults, VI - Analysis of Data and Final Report, VII Recommendations for Management and Recovery.

JUSTIFICATION

1. Problem and Need

The American burying beetle, Nicrophorus americanus Oliver, once occurred widely across the eastern two-thirds of North America. However, during the past several decades, the species has apparently suffered precipitous range and population declines (Anderson 1982; Schweitzer and Master 1987). Currently, relictual populations remain in Rhode Island (known from Block Island and recently introduced to Penikese Island), Nebraska, western Arkansas and eastern Oklahoma (Schweitzer and Master 1987; C. Raithel, unpubl. MS). The species has been documented in ten Oklahoma counties (Creighton et al. 1991; K. Frazier, pers. comm. 1993).

The Block Island population of American burying beetles has been studied intensively by biological researchers since the mid-1980's (Schweitzer and Master 1985; Kozol et al. 1987, 1988). In contrast, data on beetle distribution in Oklahoma has only been collected systematically for the past four years, with preliminary work on habitat preferences begun in 1991 (Melhop-Cifelli 1990; Creighton et al. 1991).

2. Results and Benefits Expected

Protection and recovery efforts are dependent upon accurate information about species distribution and habitat requirements. This study will provide data that will be useful for future protection and recovery efforts for this endangered species as outlined in the Recovery Plan (Raithel 1991). In addition, the genetic comparison of the Rhode Island and Oklahoma beetle populations is necessary for developing and prioritizing recovery actions for the species.

STATUS

The American burying beetle was listed as a federal and state endangered species in 1989 (USFWS, 1989). Although once distributed widely throughout most of the east from South Dakota to southern Maine and south to Texas and Florida, the species has apparently almost disappeared during the last several decades (Anderson 1982; Schweitzer and Master 1987; Raithel 1991). The species is currently known from six natural locations in four states. The largest known population occurs on Block Island, Rhode Island (Kozol et al. 1988). Single specimens were collected in Nebraska in 1989 and 1992, and approximately 25 in 1994 (C. Raithel, in litt.; K. Frazier, pers. comm.). At the start of this study, American burying beetles were known from only 2 counties in eastern Oklahoma: Latimer and Sequoyah (Creighton et al. 1991).

PROCEDURES

I. Field Surveys; Surveys will be conducted in at least six counties not surveyed in previous years. Surveys will continue to the west, north, and south of known populations until it appears that the boundaries of the species in Oklahoma has been reached. At each survey site, the habitat will be characterized (vegetation, soils and physical features). By comparing sites with and without beetles, we will improve our understanding of the beetle's habitat affinities.

II. Conduct Habitat Analysis at Each Site of Occurrence. Describe the vegetation, topography, elevation and soil type at each site of occurrence of American Burying Beetles in Oklahoma. Species composition and vegetative parameters (e.g., percent understory ground cover, vegetation height and percent canopy cover) will be recorded for the 5 m^2 area surrounding the pitfall trap where a beetle is collected, along with general habitat including proximity to open fields and forests. We will also record the presence of other burying beetles at each trap site.

III. Captive Rearing of Broods. The field experiment described in section 5 below will require captive rearing to produce offspring for transplantation from the lab to appropriate sites. The procedures will be similar to those performed in previous years. Remove adults to the laboratory for captive breeding. Cull from the larval brood a small sample to mimic natural brood culling by adult beetles (Adults cull brood members to adjust brood numbers to the size and quality of the food resource). After rearing the remainder of the brood, release the surviving progeny at the site of parental capture. Techniques for brood rearing will follow those outlined by A. Kozol at Boston University (pers. comm.), who is maintaining a captive colony of N. americanus obtained from Rhode Island. Successful brood rearing of N. orbicollis from Latimer County, Oklahoma has been accomplished during the past year. A permit has been obtained from the U.S. Fish and Wildlife Service.

IV. Collection of Beetles for Genetic Comparison Between Populations. Mitochondrial DNA analyses have been completed and we do not expect additional specimens will be required. A. Kozol has submitted the final report of these studies to M. Amaral.

V. Breeding, Habitat, and Interspecific Competition.
N. americanus search for carcasses for two reasons - to maintain their energy stores and to breed. While results of studies conducted at Fort Chaffee (Arkansas) and Camp Gruber (Oklahoma) indicate that individuals of N. americanus may be more generalized in their search for carcasses than other species, their distributions are not independent of habitat conditions. That is, even at these sites with high

densities and low diversity of habitats, N. americanus exhibited significant, albeit moderate habitat selectivity (Lomolino et al., 1994). Moreover, their ability to bury, maintain and successfully breed on carcasses may vary substantially among habitats. Anderson (1982) suggested that N. americanus requires deep, humic soils of forests. We conducted a field experiment to test this hypothesis by introducing breeding pairs onto carcasses in two habitats - forests and grasslands. Differences in breeding success between habitats were determined by comparing the number of carcasses buried and the number of offspring per carcass.

VI. Analysis of Data and Annual Report. The location and habitat type of all individuals captured will be reported. The relationships between beetle occurrence and the independent habitat variables (e.g., percent ground cover, percent canopy cover and soil type) will be analyzed using various statistical procedures. An attempt will be made to assess the habitat requirements of the species based upon these analyses. The final report will include: habitat descriptions of all sites sampled; description of potential interspecific associations; a habitat requirement assessment for the Oklahoma population.

VII. Provide Management and Recovery Recommendations. We will summarize information on the distributions and ecology of American Burying Beetles in Oklahoma and provide a list of recommendations to facilitate the recovery of this species.

I. FIELD SURVEYS FOR POPULATIONS OF N. AMERICANUS

1) Methods:

We used live trapping with baited pitfall traps to survey for the American burying beetle. Each transect was composed of eight traps spaced 20 m apart. Each pitfall trap was baited with rotted chicken and covered with a plastic dome to prevent loss of beetles due to excess heat or accumulation of rain water (see manual by Creighton et al. 1993, Appendix). Transects were located in a diversity of habitats to facilitate future characterization of habitat use by the American burying beetle.

Trapping was conducted at 179 sites across 20 counties in eastern and central Oklahoma. Trapping effort totaled to 4,232 trapnights (Table 1). The distribution of our trapping effort in Oklahoma is illustrated in Figure 1. Trapping locations are indicated by the circles in Figure 1, which were shaded proportional to the relative densities of N. americanus at those locations. Relative densities were calculated by dividing trapping success (number of N. americanus captured per functional trap night) at a particular location by the maximum trapping success at all locations.

Table 1. Legal descriptions of sites surveyed during 1991-94.

County & Site #	Legal Description	Habitat Description
Adair (Cookson Hills WMA) - 1994		
1	T14N R24E Sect. 6 NW/4 of SW/4 of SE/4	Forest
2	T14N R24E Sect. 5 NW/4 of SE/4 of SE/4	Grassland
3	T14N R24E Sect. 7 NE/4 of NW/4 of SW/4	Forest
4	T14N R24E Sect. 7 SE/4 of SW/4 of SE/4	Forest
5	T14N R24E Sect. 6 SW/4 of NW/4 of SW/4	Forest
Alfalfa (Kegelman Auxiliary Airfield) - 1993		
1	T26N R9W Sect. 13 SW/4 of NE/4	Grassland
2	T26N R9W Sect. 13 NE/4 of SW/4	Grassland
3	T26N R9W Sect. 13 NW/4 of NE/4	Grassland
4	T26N R9W Sect. 13 S/2 of SE/4	Shrubland
5	T26N R9W Sect. 12 SW/4 of NE/4	Shrubland
6	T26N R9W Sect. 12 SE/4 of NE/4	Shrubland
7	T26N R9W Sect. 12 W/2 of NW/4	Forest
8	T26N R9W Sect. SW/4 of NE/4	Forest
9	T26N R9W Sect. 13 NE/4 of NE/4	Forest
Atoka (Atoka WMA, McGee Ck. WMA, Stringtown WMA) - 1993		
1	T1S R13E Sect. 16 NE/4 of SE/4 of NW/4	Hardwood forest
2	T1S R13E Sect. 15 SW/4 of SW/4 of SW/4	Hardwood forest
3	T1S R13E Sect. 25 SE/4 of SW/w of SE/4	Rocky scrub-oak hillside
4	T1S R14E Sect. 31 NW/4 of SW/4 of NE/4	Oak-pine forest
5	T2S R14E Sect. 6 SE/4 of NE/4 of NE/4	Oak forest
6	T2S R14E Sect. 7 NE/4 of NW/4 of NW/4	Rocky oak-pine hillside
7	T2S R13E Sect. 13 NE/4 of NE/4 of NE/4	Hardwood-pine forest
8	T2S R13E Sect. 24 NE/4 of NE/4 of NE/4	Grassy scrub-oak forest
9	T2S R13E Sect. 25 NE/4 of NE/4 of NE/4	Grassy scrub-oak savannah
10	T1N R12E Sect. 26 NW/4 of SW/4 of NW/4	Bottomland hardwood forest
11	T1N R12E Sect. 24 SE/4 of SW/4 of NE/4	Rocky hardwood hillside
12	T1N R12E Sect. 23 SE/4 of SE/4 of SE/4	Rocky oak hillside
13	T1N R12E Sect. 24 NE/4 of NW/4 of NE/4	Hardwood forest
14	T1N R12E Sect. 36 NE/4 of NE/4 of NW/4	Oak forest
15	T1N R12E Sect. 24 SE/4 of SW/4 of SE/4	Grassland
16	T1N R13E Sect. 19 SW/4 of SW/4 of NE/4	Bottomland oak forest
Cherokee (Cherokee WMA) - 1994		
1	T15N R21E Sect. 23 SW/4 of NW/4 of NW/4	Grassland savannah
2	T15N R21E Sect. 15 SE/4 of SE/4 of SE/4	Hardwood forest
3	T15N R21E Sect. 15 SW/4 of SW/4 of SW/4	Hardwood forest
4	T15N R21E Sect. 21 SE/4 of SE/4 of NE/4	Grassland
5	T15N R21E Sect. 21 NE/4 of SE/4 of SE/4	Hardwood forest
6	T15N R21E Sect. 21 SW/4 of SW/4 of SE/4	Hardwood forest
7	T15N R21E Sect. 28 NW/4 of SE/4 of NW/4	Hardwood forest
8	T15N R21E Sect. 28 SW/4 of SW/4 of SW/4	Hardwood forest

Table 1. continued

9	T14N R21E	Sect. 4	SW/4 of SW/4 of NW/4	Grassland savannah
10	T14N R21E	Sect. 4	SW/4 of SW/4 of SW/4	Hardwood forest

Choctaw (Hugo WMA) - 1994

1	T5S R17E	Sect. 34	NE/4 of SE/4 of NE/4	Grassy field
2	T5S R17E	Sect. 12	NW/4 of NW/4 of NW/4	Grassland
3	T5S R17E	Sect. 1	SW/4 of SW/4 of NE/4	Hardwood forest
4	T6S R18E	Sect. 7	SE/4 of SE/4 of NE/4	Bottomland forest

Cleveland (Lexington WMA) - 1993

1	T7N R1E	Sect. 18	NE/4 of NW/4 of SE/4	Weedy field along hedgerow
2	T7N R1E	Sect. 19	NE/4 of SE/4 of SW/4	Field
3	T7N R1E	Sect. 30	SE/4 of NW/4 of SE/4	Field
4	T7N R1E	Sect. 32	NW/4 of NW/4 of NW/4	Bottomland forest
5	T7N R1E	Sect. 29	NE/4 of SE/4 of SE/4	Grassland along pond edge
6	T7N R1E	Sect. 28	SE/4 of NE/4 of NE/4	Upland mixed forest
7	T7N R1E	Sect. 21	SW/4 of SW/4 of SW/4	Grassland
8	T7N R1E	Sect. 20	NW/4 of NE/4 of NE/4	Grassland
9	T7N R1E	Sect. 18	NE/4 of SE/4 of SE/4	Grassland & mixed forest

Delaware (Spavinaw WMA) - 1993

1	T22N R22E	Sect. 28	NE/4 of SW/4 of SE/4	Oak-pine forest
2	T22N R22E	Sect. 32	SE/4 of NE/4 of NE/4	Recently logged scrub oak
3	T22N R22E	Sect. 32	NW/4 of SW/4 of NW/4	Oak-pine forest
4	T22N R22E	Sect. 31	NW/4 of NE/4 of NW/4	Hardwood forest
5	T22N R22E	Sect. 21	SW/4 of SW/4 of NE/4	Oak forest
6	T22N R22E	Sect. 20	NE/4 of NE/4 of SW/4	Open hollow
7	T22N R22E	Sect. 19	NW/4 of NW/4 of SE/4	Grassy field
8	T22N R22E	Sect. 18	SW/4 of SW/4 of NE/4	Oak forest
9	T22N R22E	Sect. 17	NE/4 of NW/4 of NE/4	Pine-oak forest
10	T22N R22E	Sect. 22	SE/4 of SW/4 of SE/4	Oak forest

Latimer - 1991

1	T6N R21E	Sect. 13	NW/4 of NW/4 of NW/4	Grazed pasture
2	T6N R21E	Sect. 13	NW/4 of NE/4 of NW/4	grazed pasture with scattered oak
3	T6N R21E	midpoint south half of Sect. 12		Second-growth Oak forest
4	T6N R21E	center of Sect. 12		Overgrown field with blackberry
5	T6N R21E	Sect. 12	NW/4	Overgrown field with blackberry
6	T6N R21E	Sect. 1	SW/4	Grassy field with scattered plante
B	T5N R20E	Sect. 12	NE/4 of NW/4 of NW/4	Bottomland forest
H	T6N R21E	Sect. 2	W/2	Oak-pine forest
7	T6N R19E	Sect. 18		Open pine-oak forest
8	T6N R18E	Sect. 13		Secondary growth, pine-oak fores
9	T6N R18E	Sect. 13		Weedy field
10	T6N R18E	Sect. 14		Weedy field
11	T6N R18E	Sect. 11		Pine-oak forest
12	T6N R18E	Sect. 10		Pine forest
13	T6N R18E	Sect. 15		Mixed forest
14	T3N R21E	Sect. 2		Pine-oak forest with small open a

Table 1. continued

15	T3N R21E	Sect. 2	Secondary pine-oak forest
16	T3N R20E	Sect. 2	Hay meadow

Leflore - 1991

1	T3N R22E	Sect. 16	Pine-oak forest
2	T3N R22E	Sect. 6	Forest edge

Love (Hickory Ck. WMA) - 1994

1	T6S R3E	Sect. 30	Hardwood forest
2	T6S R3E	Sect. 29	Hardwood forest

Marshall (Lake Texoma Rec. Area) - 1994

1	T7S R4E	Sect. 15	Grassland savannah
2	T7S R5E	Sect. 16	Bottomland forest

Mayes (Spavinaw WMA) - 1993

1	T22N R21E	Sect. 36 SW/4 of SW/4 of NW/4	Hardwood forest
2	T22N R21E	Sect. 26 SE/4 of SE/4 of NE/4	Grassland prairie

McCurtain (Tiak District) - 1993

1	T8S R25E	Sect. 36 SW/4 of NE/4 of SW/4	Pine forest
2	T9S R26E	Sect. 6 NE/4 of NW/4 of NE/4	Pine forest near field
3	T9S R26E	Sect. 5 NE/4 of SW/4 of NE/4	Open pine forest
4	T9S R26E	Sect. 4 NE/4 of SE/4 of SW/4	Bottomland forest
5	T9S R26E	Sect. 9 NW/4 of SE/4 of NW/4	Young pine plantation
6	T9S R26E	Sect. 10 SE/4 of SW/4 of NE/4	Weedy clear-cut field
7	T9S R26E	Sect. 15 SE/4 of NE/4 of SE/4	Pine forest
8	T9S R26E	Sect. 27 NW/4 of NW/4 of NW/4	Pine-oak forest
9	T8S R26E	Sect. 33 SE/4 of NW/4 of SE/4	Pine forest
10	T8S R26E	Sect. 29 SW/4 of NW/4 of SE/4	Pine-oak forest
11	T8S R25E	Sect. 11 SE/4 of NE/4 of NE/4	Weedy clear-cut field
12	T9S R26E	Sect. 1 SE/4 of SW/4 of NE/4	Weedy clear-cut field
13	T9S R27E	Sect. 5 SW/4 of SW/4 of NE/4	Open pine forest
14	T8S R27E	Sect. 29 SW/4 of SW/4 of NE/4	Pine-oak forest
15	T8S R26E	Sect. 25 SW/4 of NW/4 of SW/4	Pine-oak forest
16	T8S R24E	Sect. 23 NE/4 of NE/4 of SE/4	Pine forest next to pasture

McCurtain (Tiak District) - 1994

1	T9S R26E	Sect. 6 NW/4 of NW/4 of NE/4	Pine-Oak forest
2	T9S R26E	Sect. 5 SW/4 of SE/4 of NW/4	Pine-Oak forest
3	T9S R26E	Sect. 5 NE/4 of NE/4 of SE/4	Pine-Oak forest
4	T9S R26E	Sect. 4 NW/4 of SE/4 of SW/4	Bottomland forest

Muskogee (Camp Gruber) - 1994

1	T15N R20E	Sect. 34 SW/4 of SW/4 of SW/4	Hardwood forest
2	T15N R20E	Sect. 34 SW/4 of NE/4 of NW/4	Hardwood forest
3	T15N R20E	Sect. 27 NW/4 of SW/4 of NE/4	Hardwood forest
4	T15N R20E	Sect. 26 NE/4 of NE/4 of NW/4	Hardwood forest
5	T15N R20E	Sect. 26 SE/4 of SE/4 of NE/4	Hardwood forest

Table 1. continued

6	T15N R20E	Sect. 35	NE/4 of NE/4 of SE/4	Bottomland forest
7	T14N R20E	Sect. 12	SW/4 of SW/4 of NW/4	Grassland w/brush
8	T14N R20E	Sect. 13	SW/4 of NW/4 of NW/4	Grassland
9	T14N R20E	Sect. 13	SW/4 of SW/4 of SW/4	Grassland

Okmulgee (Okmulgee WMA) - 1993

1	T14N R12E	Sect. 5	SW/4 of NW/4 of SE/4	Hardwood forest
2	T14N R12E	Sect. 33	SW/4 of SW/4 of SW/4	Hardwood forest
3	T14N R12E	Sect. 32	NE/4 of NE/4 of NE/4	Hardwood forest
4	T14N R12E	Sect. 29	NE/4 of NE/4 of NE/4	Hardwood forest
5	T14N R12E	Sect. 30	NW/4 of NW/4 of SE/4	Grassland prairie
6	T14N R12E	Sect. 20	SW/4 of NE/4 of SW/4	Old field
7	T14N R12E	Sect. 29	SW/4 of SW/4 of SW/4	Hardwood forest
8	T14N R12E	Sect. 31	SW/4 of SW/4 of SE/4	Hardwood forest edge
9	T14N R12E	Sect. 22	NE/4 of SE/4 of NE/4	Hardwood forest
10	T14N R12E	Sect. 22	SE/4 of NW/4 of SW/4	Old wheat field

Osage (Tall Grass Prairie) - 1993

1	T27N R8E	Sect. 2	NE/4 of NW/4 of SW/4	Grassland w/ scattered oaks
2	T27N R8E	Sect. 3	SW/4 of NE/4 of SW/4	Grassland
3	T27N R8E	Sect. 4	SE/4 of SE/4 of SW/4	Grassland
4	T27N R8E	Sect. 5	SE/4 of SE/4 of SW/4	Grassland
5	T27N R8E	Sect. 9	SE/4 of SW/4 of SE/4	Grassland
6	T27N R8E	Sect. 16	NE/4 of SE/4 of SE/4	Grassland
7	T28N R8E	Sect. 35	NW/4 of NW/4 of SW/4	Weedy field
8	T28N R8E	Sect. 26	NE/4 of NE/4 of SW/4	Grassland
9	T27N R8E	Sect. 21	NW/4 of SW/4 of NW/4	Grassland
10	T27N R8E	Sect. 17	SW/4 of SE/4 of SW/4	Grassland
11	T27N R8E	Sect. 18	SW/4 of NW/4 of NE/4	Grassland
12	T27N R8E	Sect. 22	NE/4 of SW/4 of NE/4	Grassland
13	T27N R8E	Sect. 23	NW/4 of SE/4 of SW/4	Grassland
14	T27N R8E	Sect. 26	NE/4 of SE/4 of NE/4	Grassland
15	T27N R8E	Sect. 36	SW/4 of SW/4 of NE/4	Grassland
16	T27N R8E	Sect. 2	SW/4 of NE/4 of NW/4	Scrub-oak forest
17	T27N R8E	Sect. 2	NE/4 of SW/4 of SE/4	Hardwood cross-timbers
18	T27N R8E	Sect. 12	SW/4 of NE/4 of NW/4	Grassland
19	T27N R9E	Sect. 7	SW/4 of SE/4 of NW/4	Grassland
20	T27N R9E	Sect. 7	NW/4 of SE/4 of SE/4	Grassland
21	T27N R9E	Sect. 20	NW/4 of NW/4 of NW/4	Open oak forest
22	T27N R9E	Sect. 19	SW/4 of NE/4 of SE/4	Open oak forest
23	T28N R8E	Sect. 35	NE/4 of NE/4 of NW/4	Grassland
24	T28N R8E	Sect. 36	NW/4 of NE/4 of NW/4	Grassland
25	T27N R8E	Sect. 3	NE/4 of SE/4 of SE/4	Bottomland forest

Pittsburgh (McAlester Army Ammunition Depot) - 1993

1	T5N R13E	Sect. 28	SE/4 of NW/4	Upland oak forest
2	T4N R13E	Sect. 3	NW/4 of SW/4	Riparian oak forest
3	T4N R12E	Sect. 12	SW/4 of NE/4	Riparian oak forest
4	T4N R12E	Sect. 24	NE/4 of NE/4	Hay meadow

Table 1. continued

5	T4N R12E Sect. 25 SE/4 of SE/4	Hay meadow
6	T4N R13E Sect. 21 SW/4 of SW/4	Sumac shrub
7	T4N R13E Sect. 33 SW/4 of NE/4	Upland oak forest
Pushmataha (Pushmataha WMA) - 1994		
1	T1N R19E Sect. 30 SW/4 of SW/4 of NE/4	Oak-pine forest
2	T1N R19E Sect. 24 SE/4 of SE/4 of SE/4	Oak-pine forest
3	T1N R19E Sect. 25 NW/4 of SW/4 of NW/4	Hardwood forest
4	T1N R18E Sect. 34 SW/4 of NE/4 of NW/4	Hardwood forest
5	T1S R18E Sect. 5 NE/4 of NE/4 of NE/4	Grassy savannah
6	T1S R18E Sect. 4 SE/4 of SE/4 of NE/4	Hardwood forest
7	T1S R18E Sect. 10 NW/4 of SE/4 of NE/4	Hardwood creek bottom
8	T1S R18N Sect. 12 NE/4 of SW/4 of NW/4	Hardwood-pine forest
9	T1S R18E Sect. 1 SW/4 of SE/4 of NE/4	Hardwood-pine forest
10	T1N R19E Sect. 31 NW/4 of SW/4 of NW/4	Hardwood-pine forest
Sequoyah - 1991		
1	T13N R22E Sect. 5 NW/4 of NE/4 of NW/4	Bottomland forest
2	T13N R22E Sect. 5 NW/4 of NW/4 of NW/4	Post oak forest
3	T13N R22E Sect. 6 NE/4 of SE/4 of NW/4	Post oakforest on steep hillside
4	T13N R22E Sect. 7 NW/4 of NE/4 of NW/4	Open oak forest along edge of fie
5	T13N R21E Sect. 13 NW/4 of NE/4 of NW/4	Grassland
6	T13N R21E Sect. 12 NE/4 of SE/4 of NE/4	open post oak forest
Wagoner (Fort Gibson WMA) - 1994		
1	T18N R18E Sect. 9 SW/4 of SW/4 of SW/4	Grassland
2	T18N R18E Sect. 3 SE/4 of SE/4 of SE/4	Grassland
3	T18N R18E Sect. 16 SE/4 of NE/4 of NE/4	Forest

2) Results

A total of 6,374 burying beetles of 7 species was live-trapped during these studies (Table 2). Only 207 (3.2%) of these captures, however, were N. americanus. In comparison to N. americanus, N. orbicollis was over 20 times more abundant, while N. tomentosus was nearly 5 times as abundant and N. marginatus were over twice as abundant. Three species, N. pustulatus, N. sayi and N. carolinus were less common than N. americanus.

N. americanus were detected in 9 of the 20 counties surveyed. The highest population densities of N. americanus (trapping success = 47.6%) were recorded in Muskogee County (Camp Gruber). In contrast, trapping success of N. americanus in McCurtain County was only 18.6%, i.e., less than half that recorded in Muskogee County. All other counties recorded lower relative densities of N. americanus (trapping success $\leq$ 11%).

The geographic distribution of captures and relative densities of N. americanus (Figure 1) indicated that the range of N. americanus populations in Oklahoma includes the central-eastern to south-eastern portions of the state. We suspect that individuals may occasionally be detected outside this range, but they will probably represent extra-limital individuals dispersing from source populations within the estimated range (Figure 1).

II. ANALYSIS OF HABITAT AFFINITIES

1) Methods

Analyses of habitat affinities of N. americanus were restricted to those sites occurring within counties included in the estimated range of N. americanus (Figure 1). Habitat sampling methods used in the current studies are described in the manual entitled "Survey Methods for the American Burying Beetle (N. americanus) in Oklahoma and Arkansas," by Creighton et al., 1993 (see Appendix). Habitat variables recorded at each trap station (Table 3) were subjected to Principle Components Analysis (using correlation matrices; SYSTAT, 1992) to describe habitat characteristics in terms of three new, orthogonal variables derived from combinations of the original habitat variables (canopy closure, aspect, soil depth, ground cover, etc.) The three derived variables (Factor scores, Table 4) accounted for 57.8% of the variance in habitat variables recorded in the field. Factor 1, which accounted for 29.4% of the total variance, was basically a measure of forest development (loading strongly on canopy closure, distance to open area and litter accumulation). Factor 2, which accounted for 17.3% of the total variance, was a measure of soil depth. Factor 3, which accounted for 11.1% of the total variance, was a measure of understory woody cover, primarily by small shrubs.

The three Factor scores were then used in a KMEANS cluster analysis (SYSTAT, 1992) to assign each trap site to one of 10 habitat categories.

Figure 1.
Distribution and relative densities of the American Burying Beetle in Oklahoma, 1991 to 1994. Circles indicate survey locations. Shading is proportional to the relative densities at each location. Dashed line represents estimated range boundary in Oklahoma. Individuals may be reported outside this range, but they will likely represent dispersers from source population within the estimated range.

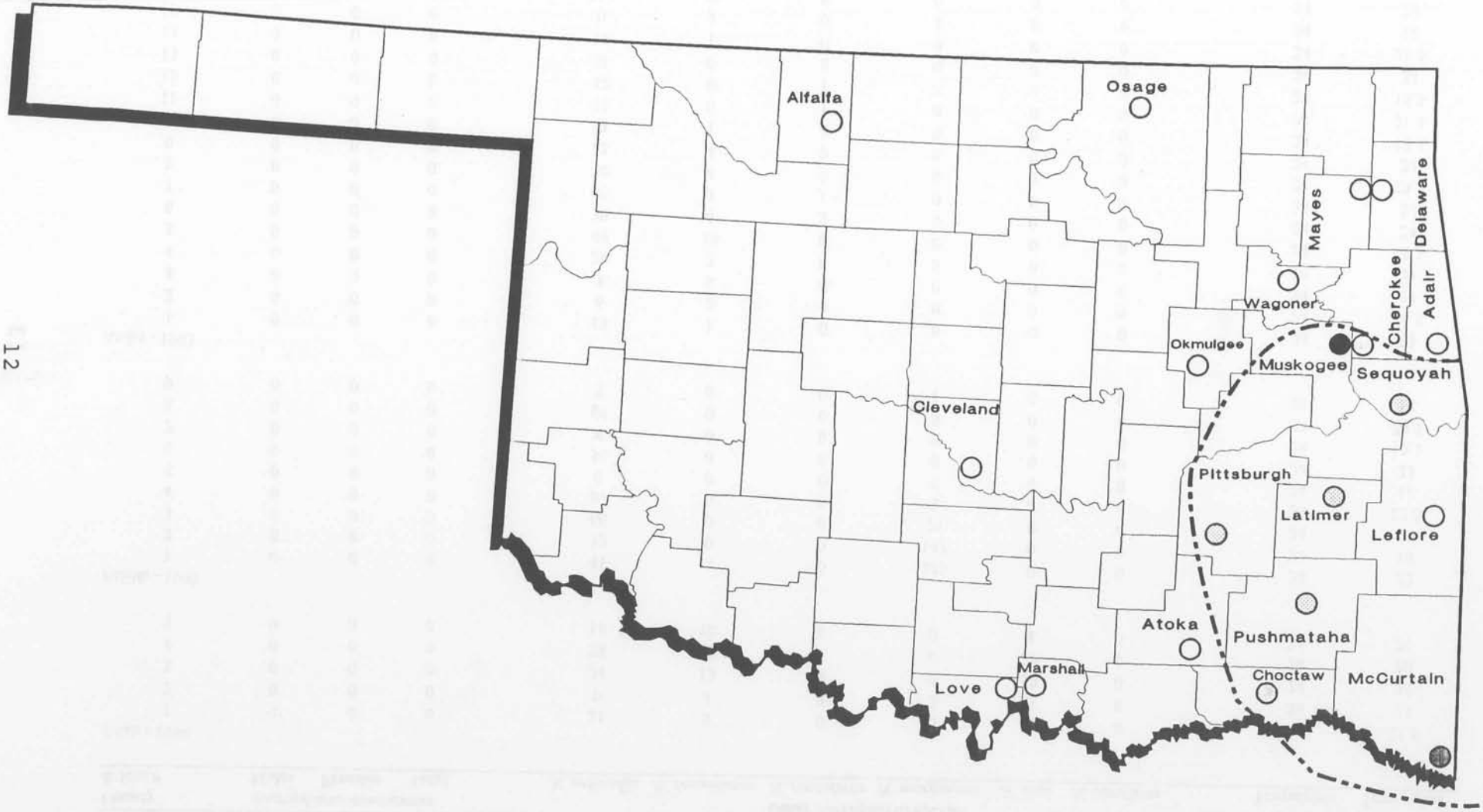


Table 2. Summary of results for trapping during 1991-94

County & Site #	*Nicrophorus americanus* Males	Females	Total	Other Nicrophorus species *N. orbicollis*	*N. tomentosus*	*N. pustulatus*	*N. marginatus*	*N. sayi*	*N. carolinus*	Total Trapnights	Functional Trapnights
Adair - 1994											
1	0	0	0	21	9	0	0	16	0	24	21.5
2	0	0	0	0	4	0	2	0	0	24	23
3	0	0	0	24	13	0	0	12	0	24	24
4	0	0	0	28	20	0	0	29	0	24	20
5	0	0	0	16	18	1	0	4	0	24	20
Alfalfa - 1993											
1	0	0	0	41	0	0	127	0	0	24	22
2	0	0	0	52	0	0	147	0	0	24	18
3	0	0	0	66	0	0	25	0	0	24	24
4	0	0	0	64	0	0	8	0	0	24	22.5
5	0	0	0	0	0	0	0	0	0	24	12
6	0	0	0	50	0	0	0	0	0	24	23
7	0	0	0	8	0	0	2	0	0	24	18.5
8	0	0	0	67	0	0	0	0	0	24	20.5
9	0	0	0	7	0	0	1	0	0	24	18
Atoka - 1993											
1	0	0	0	19	1	0	0	0	0	24	23
2	0	0	0	6	2	0	0	0	0	24	16.5
3	0	0	0	3	0	0	0	0	0	24	24
4	0	0	0	29	2	0	0	0	0	24	24
5	0	0	0	49	13	0	0	0	0	24	23.5
6	0	0	0	30	4	2	0	0	0	24	24
7	0	0	0	60	3	1	0	0	0	24	24
8	0	0	0	20	4	0	0	0	0	24	23.5
9	0	0	0	15	4	0	0	0	0	24	24
10	0	0	0	31	1	0	0	0	0	24	17.5
11	0	0	0	12	0	1	0	0	0	24	21.5
12	0	0	0	42	0	1	0	0	0	24	15.5
13	0	0	0	51	0	8	0	0	0	24	24
14	0	0	0	74	1	0	0	0	0	24	21.5
15	0	0	0	0	4	0	3	0	0	24	24
16	0	0	0	216	0	9	0	0	0	24	24
Cherokee - 1994											
1	0	1	1	4	9	0	0	0	0	24	22
2	1	1	2	33	29	0	0	11	0	24	23
3	0	0	0	7	3	0	0	5	0	24	22.5
4	1	0	1	0	4	0	0	0	0	24	23
5	2	0	2	0	2	0	0	1	0	24	23.5
6	1	2	3	3	10	0	0	2	0	24	24

Table 2. continued

7	0	0	0	11	2	0	0	2	0	24	19.5
8	0	3	3	1	0	0	0	0	0	24	22.5
9	0	0	0	0	3	0	0	0	0	24	23.5
10	0	0	0	28	11	0	0	0	0	24	23.5
Choctaw - 1994											
1	0	0	0	0	0	0	0	0	2	24	21
2	0	1	1	0	0	0	1	0	15	24	23
3	1	2	3	21	0	0	0	0	0	24	22
4	0	0	0	6	0	0	0	0	0	24	12.5
Cleveland - 1993											
1	0	0	0	2	0	0	0	0	0	24	23.5
2	0	0	0	2	1	0	1	0	0	24	24
3	0	0	0	1	0	0	0	0	0	24	22
4	0	0	0	59	0	0	0	0	0	24	21
5	0	0	0	0	0	0	0	0	0	24	23
6	0	0	0	35	0	0	0	0	0	24	15.5
7	0	0	0	0	2	0	0	0	0	24	24
8	0	0	0	0	0	0	0	0	0	24	24
9	0	0	0	11	0	0	0	0	0	24	22.5
Delaware - 1993											
1	0	0	0	58	1	0	0	0	0	24	24
2	0	0	0	4	0	0	0	0	0	24	24
3	0	0	0	39	2	0	0	0	0	24	24
4	0	0	0	140	4	0	0	0	0	24	21.5
5	0	0	0	34	1	1	0	0	0	24	24
6	0	0	0	40	3	1	0	0	0	24	24
7	0	0	0	20	3	0	2	0	0	24	23
8	0	0	0	129	3	2	0	0	0	24	23.5
9	0	0	0	46	3	0	0	0	0	24	23.5
10	0	0	0	19	3	4	0	0	0	24	24
Latimer - 1991											
1	0	0	0	7	0	1	1	0	0	48	46
2	0	0	0	19	0	0	0	0	0	48	44.5
3	0	1	1	49	3	5	0	0	0	48	45.5
4	0	1	1	29	2	0	0	0	0	48	42
5	1	0	1	75	0	6	0	0	0	48	42
6	0	1	1	1	1	0	4	0	0	48	44.5
B	0	0	0	47	1	0	0	0	0	32	27
H	1	1	2	35	8	2	0	0	0	32	29.5
7	0	0	0	23	0	0	0	0	0	12	11.5
8	0	0	0	35	0	0	0	0	0	18	16
9	0	0	0	2	0	0	0	0	0	18	10.5
10	0	0	0	1	0	0	0	0	0	18	17.5
11	0	0	0	35	0	1	0	0	0	18	16

Table 2. continued

12	0	0	0	33	0	0	0	0	0	12	11.5
13	0	0	0	54	0	0	0	0	0	18	16.5
14	0	0	0	90	0	0	0	0	0	18	18
15	0	0	0	30	0	1	0	0	0	18	17
16	0	0	0	14	0	0	0	0	0	18	14.5
Leflore - 1991											
1	0	0	0	30	0	0	0	0	0	18	18
2	0	0	0	52	0	0	0	0	0	18	17
Love - 1994											
1	0	0	0	4	4	0	0	0	2	24	23.5
2	0	0	0	0	0	0	0	0	0	24	24
Marshall - 1994											
1	0	0	0	0	5	0	0	0	0	24	24
2	0	0	0	8	7	0	0	0	12	24	15
Mayes - 1993											
1	0	0	0	32	5	1	0	0	0	24	20.5
2	0	0	0	1	3	0	2	0	0	24	24
McCurtain - 1993											
1	1	0	1	11	18	0	0	0	0	24	24
2	**4**	**6**	**10**	**88**	**6**	**0**	**0**	**0**	**0**	**24**	**24**
3	**6**	**12**	**18**	**29**	**5**	**0**	**0**	**0**	**0**	**24**	**24**
4	0	0	0	78	15	0	0	0	0	24	23.5
5	0	0	0	36	5	0	0	0	0	24	18.5
6*	0	0	0	0	0	0	0	0	0	24	24
7	2	1	3	33	5	0	0	0	0	24	24
8	0	1	1	21	2	0	0	0	0	24	19
9	0	0	0	12	6	0	0	0	0	24	21
10	1	0	1	24	6	0	0	0	0	24	22.5
11*	0	0	0	0	0	0	0	0	1	24	23
12*	1	0	1	1	4	0	0	0	0	24	23
13	0	1	1	21	18	0	0	0	0	24	23.5
14	1	0	1	82	25	0	0	0	0	24	18.5
15	1	0	1	32	9	0	0	0	0	24	10
16	0	1	1	42	0	0	0	0	0	24	20.5
McCurtain - 1994											
1	1	1	2	28	2	0	0	0	0	24	23.5
2	**10**	**13**	**23**	**20**	**3**	**2**	**0**	**0**	**0**	**24**	**23.5**
3	**6**	**10**	**16**	**34**	**3**	**1**	**0**	**0**	**0**	**24**	**23**
4	0	1	1	106	0	1	0	0	0	24	22
Muskogee - 1994											
1	2	0	2	5	11	0	0	1	0	24	24

Table 2. continued

2	11	14	25	6	12	1	0	6	0	24	24
3	9	16	25	1	6	0	0	3	0	24	24
4	3	1	4	2	8	0	0	4	0	24	21.5
5	4	3	7	5	2	0	0	4	0	24	24
6	1	5	6	9	12	0	0	3	0	24	12
7	4	2	6	0	9	0	1	0	0	24	23.5
8	6	11	17	0	23	0	9	1	0	24	22
9	2	0	2	0	6	0	0	0	0	24	22.5
Okmulgee - 1993											
1	0	0	0	5	0	0	0	0	1	24	22
2	0	0	0	2	1	0	0	0	1	24	19
3	0	0	0	2	0	0	0	0	0	24	20
4	0	0	0	3	0	0	0	0	1	24	21.5
5	0	0	0	0	0	0	1	0	3	24	11
6	0	0	0	0	0	0	1	0	2	24	15.5
7	0	0	0	5	0	1	0	0	1	24	19.5
8	0	0	0	7	1	0	0	0	0	24	16
9	0	0	0	6	0	0	0	0	0	24	19.5
10	0	0	0	1	0	0	0	0	9	24	22
Osage - 1993											
1	0	0	0	12	4	0	1	0	0	24	23.5
2	0	0	0	0	7	0	4	0	0	24	19
3	0	0	0	0	13	0	10	0	0	24	24
4	0	0	0	0	0	0	8	0	0	24	22
5	0	0	0	0	8	0	9	0	0	24	23
6	0	0	0	0	5	0	6	0	0	24	20
7	0	0	0	3	4	0	7	0	0	24	21.5
8	0	0	0	0	13	0	15	0	0	24	23
9	0	0	0	0	9	0	49	0	0	24	22
10	0	0	0	0	10	0	16	0	0	24	19
11	0	0	0	0	1	0	34	0	0	24	19
12	0	0	0	1	1	0	7	0	0	24	16
13	0	0	0	0	9	0	14	0	0	24	21
14	0	0	0	0	3	0	11	0	0	16	12
15	0	0	0	0	6	0	10	0	0	24	20.5
16	0	0	0	66	24	0	0	0	0	24	24
17	0	0	0	113	52	2	0	0	0	24	23.5
18	0	0	0	5	36	0	9	0	0	24	12
19	0	0	0	0	11	0	5	0	0	24	16
20	0	0	0	0	6	0	7	0	0	24	23
21	0	0	0	28	73	0	0	0	0	24	18.5
22	0	0	0	11	35	1	0	0	0	24	16.5
23	0	0	0	2	17	0	4	0	0	24	22
24	0	0	0	1	18	0	8	0	0	24	21.5
25	0	0	0	206	45	0	0	0	0	24	21.5

Table 2. continued

Pittsburgh - 1993											
1	0	1	1	30	0	0	1	0	0	24	20.5
2	0	0	0	9	0	1	0	0	0	24	17
3	0	0	0	1	0	0	0	0	0	24	23.5
4	0	0	0	0	0	0	0	0	0	24	24
5	0	0	0	0	0	0	0	0	0	24	24
6	1	1	2	6	0	0	2	0	0	24	23.5
7	0	0	0	10	0	0	0	0	0	24	14.5
Pushmataha - 1994											
1	1	0	1	31	6	0	0	1	0	24	24
2	0	0	0	30	3	0	0	0	0	24	22.5
3	0	0	0	38	12	0	0	1	0	24	24
4	0	0	0	57	14	0	0	1	0	24	23.5
5	1	0	1	11	8	0	0	0	0	24	24
6	0	0	0	28	3	1	0	0	0	24	23.5
7	1	1	2	160	10	0	0	2	0	24	24
8	0	0	0	19	15	0	0	0	0	24	24
9	0	0	0	86	14	0	0	1	0	24	24
10	2	0	2	59	5	0	0	0	0	24	24
Sequoyah - 1991											
1	0	0	0	13	10	0	0	0	0	12	8.5
2	0	0	0	0	3	0	0	0	0	12	12
3	0	0	0	7	2	0	0	0	0	12	11.5
4	0	0	0	13	6	0	0	0	0	12	12
5	1	0	1	0	2	0	0	0	0	16	15.5
6	0	0	0	6	6	0	0	0	0	12	12
Wagoner - 1994											
1	0	0	0	1	0	0	0	0	0	24	24
2	0	0	0	0	0	0	0	0	0	24	23.5
3	0	0	0	0	0	0	0	0	0	24	12
Totals 179	**91**	**116**	**207**	**4380**	**993**	**59**	**575**	**110**	**50**	**4296**	**3856**

* Indicates sites in McCurtain Co. that have been clearcut.
Bold print in McCurtain Co. indicates sites that are intended to be cut.

Table 3. Habitat data for sites surveyed during 1991-1994.

County & Site #	Mean Soil Depth (dm)	Mean Canopy Openness (%)	% Slope	Aspect	Distance to Forest (m)	Distance to Open (m)	Grass	Herb	Litter	Rock	Shrub < 6 ft.	Shrub > 6 ft	Tree	Moss
Adair - 1994														
1	2.83	0.08	13	120	0	100	6	6	7	2	1	0	2	3
2	0.50	96.00	9	140	60	0	7	7	0	2	0	0	0	0
3	2.17	0.33	22	280	0	>180	3	5	7	7	0	0	0	7
4	1.50	3.75	25	220	0	>180	4	4	7	6	3	0	0	4
5	0.50	1.50	15	210	0	95	4	7	7	5	5	0	1	3
Alfalfa - 1993														
1	3.33	96.00	2	137	>180	0	7	6	0	0	2	0	0	0
2	3.67	96.00	3	166	>180	0	7	7	0	0	1	0	0	0
3	4.00	96.00	3	143	20	0	7	7	0	0	5	0	0	0
4	3.33	96.00	0	0	>180	0	7	7	0	0	0	0	0	0
5	3.50	9.67	40	62	0	35	5	6	5	0	6	0	1	0
6	3.67	96.00	5	84	20	0	7	7	0	0	5	0	0	0
7	3.30	32.33	9	120	0	80	7	3	1	0	2	0	0	0
8	3.83	96.00	1	26	50	0	7	7	1	0	4	0	0	0
9	4.00	8.92	3	171	0	20	5	7	7	0	3	0	0	0
Atoka - 1993														
1	1.17	13.25	10	265	0	>180	7	7	7	4	3	0	0	0
2	1.50	22.33	12	230	0	>180	6	7	7	6	2	0	1	2
3	0.50	35.50	20	165	0	>180	7	5	7	3	0	0	0	1
4	2.17	11.17	5	70	0	>180	3	6	7	3	1	0	3	1
5	4.17	19.83	5	8	0	>180	6	6	7	5	5	0	0	1
6	0.50	39.75	10	190	0	>180	6	4	7	4	2	0	1	1
7	4.50	35.83	2	110	0	20	5	6	7	0	4	0	0	2
8	4.50	24.25	3	130	0	0	7	4	7	0	2	0	0	0
9	4.50	90.67	5	85	0	0	7	7	7	0	2	0	0	0
10	0.50	0.00	6	140	0	>180	5	7	7	0	7	0	0	0
11	0.50	1.33	28	270	0	>180	4	6	7	6	4	0	0	0
12	0.50	0.00	22	300	0	>180	0	7	7	7	3	0	0	2
13	3.50	14.08	5	140	0	>180	7	7	7	1	2	0	2	3
14	1.17	0.92	4	180	0	>180	7	7	7	4	4	0	0	1
15	2.17	96.00	5	180	60	0	7	7	0	0	0	0	0	0
16	3.50	1.08	2	145	0	>180	7	7	7	0	5	0	0	0
Cherokee - 1994														
1	2.17	62.17	5	50	50	0	6	7	2	1	2	0	0	0
2	1.83	11.33	8	23	0	>180	6	7	7	4	4	0	0	4
3	2.83	44.33	4	240	10	0	7	6	7	2	3	0	0	3

Table 3. continued

4	3.17	96.00	3	340	60	0	7	7	0	0	0	0	0	5
5	1.83	61.17	4	140	0	>180	7	7	7	3	2	0	0	0
6	1.17	47.58	4	130	0	>180	7	7	7	1	2	0	0	0
7	4.50	2.83	1	255	0	>180	7	7	7	0	3	0	0	3
8	0.50	18.67	8	270	0	80	4	7	7	7	0	0	0	0
9	1.83	70.75	2	130	0	0	7	7	6	0	3	0	0	0
10	1.17	1.33	1	100	0	>180	6	6	7	4	6	0	0	1
Choctaw - 1994														
1	4.50	95.17	3	90	60	0	7	7	1	0	2	0	0	1
2	1.83	96.00	1	190	70	0	7	7	0	0	1	0	0	5
3	4.50	2.33	3	24	0	50	7	7	7	0	5	0	0	1
4	4.17	25.67	4	100	0	50	6	7	7	0	6	0	1	2
Cleveland - 1993														
1	4.50	61.08	4	250	<15	0	6	7	5	0	2	1	1	3
2	4.17	95.75	3	150	<15	0	7	7	4	0	1	0	0	1
3	4.17	93.00	2	110	0	0	7	6	4	0	1	0	0	2
4	4.17	0.42	7	85	0	5	6	7	7	0	3	0	0	0
5	4.17	68.42	6	98	0	0	7	7	5	0	1	1	0	5
6	4.50	3.25	8	0	0	>100	2	7	7	0	4	0	0	0
7	3.83	96.00	5	356	30.7	0	7	7	0	0	1	0	0	0
8	4.17	94.00	4	359	5	0	7	7	4	0	5	2	0	5
9	4.50	48.17	5	258	0	2	6	7	7	1	4	0	0	1
Delaware - 1993														
1	0.50	5.75	15	330	0	>180	0	7	7	1	0	0	1	3
2	0.50	81.83	5	45	0	0	6	6	7	5	2	2	0	0
3	0.50	14.33	5	225	0	40	6	6	7	7	1	0	0	3
4	0.50	5.83	3	100	0	>180	3	4	7	2	3	0	0	0
5	0.50	0.83	22	242	0	>180	0	2	7	7	5	1	0	2
6	0.50	81.58	1	310	30	0	0	7	2	5	0	0	0	0
7	0.50	96.00	1	220	55	0	7	5	2	7	0	0	0	4
8	1.83	0.75	19	300	0	>180	0	5	7	7	4	1	0	1
9	0.50	0.17	20	350	0	>180	0	5	7	7	1	0	0	1
10	0.50	9.42	10	150	0	>180	4	4	7	7	1	0	0	1
Latimer - 1991														
1	0.50	60.50	6	90	0	0	7	7	3	2	0	0	0	2
2	0.50	1.83	15	155	0	100	0	4	7	4	3	0	0	0
3	0.50	33.50	10	295	0	10	7	6	5	5	3	0	0	1
4	0.50	73.50	2	50	0	10	7	7	7	4	6	0	0	0
5	0.50	18.08	3	120	0	60	6	7	6	4	5	0	0	3
6	3.17	96.00	3	170	70	0	7	7	0	0	2	0	0	0
B	1.17	2.33	7	165	0	70	7	7	7	0	6	0	0	0

Table 3. continued

H	1.17	0.00	6	75	0	100	5	7	7	5	4	0	1	2
Leflore - 1991														
1														
2														
Love - 1994														
1	1.83	33.83	8	235	0	10	6	7	7	0	2	0	0	1
2	4.50	35.17	10	22	0	0	7	7	7	0	2	0	0	3
Marshall - 1994														
1	2.50	96.00	5	349	50	0	7	7	1	0	4	0	0	0
2	3.83	0.17	5	2	0	>180	5	7	7	0	4	1	1	2
Mayes - 1993														
1	0.83	21.25	0	300	0	5	7	7	7	5	2	0	0	1
2	4.50	96.00	1	220	25	0	7	7	0	0	0	0	0	0
McCurtain - 1993														
1	3.50	10.83	0	0	0	>180	4	5	7	0	6	0	0	0
2	3.83	3.92	3	190	0	80	2	7	7	0	3	0	1	0
3	4.17	11.00	0	0	0	70	4	7	7	0	2	0	0	0
4	4.50	0.17	3	260	0	>180	5	5	7	0	3	0	0	2
5	4.17	0.00	3	50	0	>180	0	7	7	0	1	4	3	2
6	2.50	96.00	0	0	100	0	7	7	1	0	6	0	0	0
7	4.17	6.75	0	0	0	>180	6	7	7	0	6	0	1	1
8	4.17	0.92	1	40	0	>180	1	4	7	0	5	0	2	1
9	4.17	4.08	3	60	0	>180	7	7	7	0	5	0	0	0
10	2.50	8.33	5	250	0	>180	7	7	7	0	4	0	0	4
11	3.50	96.00	0	0	100	0	7	7	3	0	6	0	0	2
12	3.50	96.00	0	0	75	0	7	7	3	0	2	0	0	1
13	4.50	5.42	0	0	0	90	7	7	7	0	5	0	0	1
14	4.17	0.67	5	10	0	>180	5	7	7	0	3	0	0	0
15	3.83	2.75	0	0	0	80	5	7	7	0	3	0	1	1
16	4.50	0.25	3	280	0	80	1	7	7	0	3	0	1	0
McCurtain - 1994														
1	3.83	2.42	2	240	0	150	6	7	7	0	3	1	0	0
2	4.50	1.33	2	240	0	>180	6	7	7	0	1	1	0	0
3	4.50	1.58	3	170	0	>180	5	7	7	0	1	0	0	0
4	4.50	0.00	0	0	0	>180	6	7	7	0	5	0	0	2
Muskogee - 1994														
1	3.50	4.42	7	95	0	>180	7	7	7	3	2	0	1	1
2	2.17	2.83	7		0	150	7	7	7	5	7	0	1	2

Table 3. continued

3	2.50	14.75	5	340	0	>180	7	7	7	2	6	0	1	2
4	1.17	44.50	9	290	0	40	7	7	4	2	3	1	0	2
5	1.50	27.75	5	122	0	>180	7	4	7	1	3	0	0	3
6	4.50	1.92	8	80	0	75	6	5	7	1	1	0	0	2
7	3.17	96.00	1	330	60	0	7	7	0	0	3	0	0	0
8	3.50	96.00	0	0	>180	0	7	7	0	0	0	0	0	1
9	0.50	96.00	1	150	>180	0	7	7	7	1	5	0	0	0
Okmulgee - 1993														
1	0.50	10.50	9	182	0	>180	6	7	7	4	2	0	0	0
2	0.50	34.67	22	110	0	40	6	3	7	5	0	0	0	2
3	0.83	12.92	15	310	0	>180	7	5	7	5	2	1	0	1
4	0.50	1.50	5	165	0	20	5	7	7	0	3	0	0	0
5	1.50	96.00	5	180	>180	0	7	7	0	0	3	0	0	0
6	0.50	96.00	1	75	40	0	7	7	3	0	1	0	0	0
7	0.83	1.75	10	300	0	>180	5	6	7	1	2	0	1	2
8	1.50	34.25	2	250	0	50	5	7	7	0	1	0	1	2
9	0.50	16.08	8	275	0	75	5	5	7	0	4	0	0	1
10	2.50	90.92	3	165	30	0	7	7	0	0	0	0	0	0
Osage - 1993														
1	1.83	96.00	12	340	50	0	7	6	7	0	2	0	0	0
2	3.50	96.00	7	120	70	0	7	7	0	0	0	0	0	0
3	1.83	96.00	2	27	>180	0	7	5	0	0	0	0	0	0
4	1.17	96.00	5	170	>180	0	7	5	0	0	0	0	0	0
5	2.50	96.00	5	240	>180	0	7	7	0	1	0	0	0	0
6	3.83	96.00	5	310	>180	0	7	7	0	0	0	0	0	0
7	3.83	96.00	5	180	100	0	7	7	7	0	7	0	0	0
8	1.83	96.00	10	140	>180	0	7	7	0	2	0	0	0	0
9	3.83	96.00	15	20	>180	0	7	7	0	0	2	0	0	0
10	2.17	96.00	6	170	>180	0	7	7	0	1	0	0	0	0
11	2.83	96.00	3	10	>180	0	7	6	0	0	0	0	0	0
12	2.83	96.00	5	40	>180	0	7	7	1	1	3	0	0	0
13	2.83	96.00	10	310	>180	0	7	7	0	3	0	0	0	0
15	2.83	96.00	15	120	>180	0	7	7	0	5	1	0	0	0
16	4.17	88.08	5	240	0	>180	7	6	7	0	3	0	0	0
17	4.50	14.42	7	22	0	100	7	4	7	0	1	1	0	0
18	3.83	96.00	4	200	>180	0	7	6	0	0	3	0	0	0
19	3.50	96.00	8	270	>180	0	7	7	0	0	0	0	0	0
20	3.50	96.00	3	170	>180	0	7	7	0	1	0	0	0	0
21	2.17	32.58	12	130	0	35	7	7	6	2	0	0	0	0
22	2.50	53.17	10	230	0	0	7	7	5	5	1	0	1	0
23	4.17	96.00	5	170	>180	0	7	7	0	0	0	0	0	0
24	3.83	96.00	4	340	>180	0	7	7	0	0	0	0	0	0
25	4.17	12.33	8	190	0	30	7	7	7	0	2	0	0	0

Table 3. continued

Pittsburgh - 1993														
1	3.17	8.75	10	55	0	>180	4	6	7	5	0	0	3	0
2	1.83	2.33	7	90	0	>180	7	7	7	0	4	0	1	0
3	1.50	95.92	5	160	150	0	7	7	0	0	0	0	0	0
4	1.83	96.00	0	0	>180	0	7	7	0	0	0	0	0	0
5	2.17	96.00	8	280	>180	0	7	7	0	0	4	0	0	0
6	1.83	7.45	5	175	0	>180	7	4	7	0	3	0	2	0
7	1.50	0.08	5	40	0	20	3	7	7	0	4	0	1	0
Pushmataha - 1994														
1	0.50	22.33	11	148	0	>180	6	6	7	5	3	0	0	2
2	1.83	2.25	12	175	0	>180	4	4	7	4	4	0	0	2
3	0.83	1.50	13	145	0	>180	4	3	7	6	2	0	2	2
4	3.83	1.92	9	106	0	>180	5	5	7	6	2	0	0	3
5	0.83	58.42	2	110	8	0	7	7	4	2	1	0	0	2
6	2.00	4.67	7	10	0	>180	7	6	7	2	1	0	0	3
7	2.50	1.33	10	330	0	50	7	7	7	4	4	0	0	6
8	2.50	15.92	3	292	0	80	6	6	6	3	3	1	0	5
9	2.17	2.58	4	90	0	70	6	5	7	1	0	0	1	4
10	0.50	2.92	7	263	0	>180	7	7	6	2	1	0	0	2
Sequoyah - 1991														
1	0.83	0.00	5	320	0	>180	5	6	7	1	4	0	1	2
2	0.50	2.83	8	10	0	>180	4	7	7	7	2	0	1	1
3	1.17	0.00	30	275	0	>180	5	5	7	6	2	0	1	5
4	1.50	0.33	5	120	0	55	5	7	7	4	3	0	0	2
5	1.50	75.33	15	65	0	0	7	7	7	4	0	1	0	1
6	1.50	21.67	22	320	0	0	5	5	7	5	0	0	0	0
Wagoner - 1994														
1	4.50	96.00	0	2	50	0	7	7	0	0	0	0	0	0
2	4.50	96.00	6	165	15	0	7	7	0	0	0	0	0	0
3	4.50	12.83	1	150	0	70	5	5	7	0	0	0	0	0

Before conducting the cluster analysis, Factor scores were weighted by multiplying each by the percent variance they explained. Trap sites were then assigned to one of ten habitat categories and the total number of functional trap nights and individuals of each species were then calculated for all sites within each category (Table 5).

Table 4. Averages of standardized Factor scores for each of ten habitat clusters. Factor scores were calculated by Principal Components Analysis of habitat variables recorded at sites within counties with known records of the American burying beetles. Scores for each site were then subjected to KMEANS CLUSTER analysis to group each site into one of ten habitat clusters.

Habitat cluster	Factor 1 (forest development)	Factor 2 (soil depth)	Factor 3 (shrub cover)
1	-1.73	-0.15	-0.49
2	+0.22	+1.16	+0.34
3	+0.55	-0.11	+0.44
4	+1.34	+0.92	-1.53
5	-0.20	-0.19	+0.78
6	+1.70	-1.70	-0.85
7	+0.64	-1.91	-0.68
8	+.025	-1.31	+0.68
9	+1.03	-0.83	+0.18
10	-0.49	-1.00	-0.45

Table 5. Results of live-trapping studies for sites within each of the ten habitat clusters. Data only for those sites within counties with known records of the American burying beetle.

Habitat cluster	Functional trapnights	All ABB	Female ABB	Male ABB	CARO	MARG	ORBI	PUST	SAYI	TOME
1	343	30	16	14	18	15	7	0	1	56
2	367	81	48	33	0	0	595	5	2	102
3	321.5	47	27	20	0	2	383	3	23	148
4	39.5	2	2	0	0	1	51	0	0	2
5	204.5	7	3	4	0	0	161	6	8	21
6	80	0	0	0	0	0	64	0	1	14
7	34.5	3	3	0	0	0	7	0	0	6
8	93.5	5	2	3	0	0	268	5	2	18
9	82	1	0	1	0	0	118	0	2	26
10	107	6	1	5	0	1	20	1	4	18

Species Codes: ABB = the American burying beetle(N. americanus), CARO = N. carolinus, MARG = N. marginatus, ORBI = N. orbicollis, PUST = N. pustulatus, SAYI = N. sayi and TOME = N. tomentosus.

We then tested the null hypothesis that N. americanus and syntopic species were habitat generalists. That is, we tested the hypothesis that the distribution of these species was identical to the distribution of trapping effort across habitat clusters. We used a goodness-of-fit test comparing the observed number of individuals captured for each habitat cluster to the number expected (expected number within a particular habitat cluster = total number of individuals for the species, times the proportion of functional trap nights within that habitat cluster). We also calculated interspecific overlap among burying beetles. We used overlap measures (Ludwig and Reynolds, 1988) to calculate indices of niche breadth and interspecific overlap. These measures vary from 0.00 for a perfect specialist (complete niche segregation) to 1.00 for a perfect generalist (complete overlap).

2) Results

Habitat Associations at the Landscape Level

All species of burying beetles, including N. americanus, exhibited significant habitat selectivity at the landscape level (i.e., their niche breadths were significantly less than the maximum value of 1.00; Table 6). Trapping success (number of individuals captured per functional trapnight) of N. americanus was highest for sites in habitat clusters 2 and 3 (trapping success = 0.22 and 0.15, respectively). These sites are characterized as having moderate to well-developed forest with moderate to deep soils and an understory with moderate cover of small shrubs (Table 4). The absence of N. americanus in sites of habitat cluster 6, indicates that forest development, alone, does not constitute optimal habitat for this species. Rather, this species may require sites that combine well-developed forests with deep soils and moderate shrub cover.

N. tomentosus exhibited the broadest habitat niche (0.89), followed by N. americanus (0.78). Both of these species exhibited high overlap with each other as well as most other species of burying beetles (Table 7). In contrast, N. carolinus and N. marginatus exhibited extremely narrow habitat breadths (niche breadths = 0.21 and 0.36, respectively), both preferring habitat cluster 1, i.e., open, grassland sites with little soils and little shrub cover. N. orbicollis (niche breadth = 0.71), on the other hand, preferred forested sites with little soils, but dense shrub cover.

Generalized overlap of all species, taken simultaneously, was high (0.808 for adjusted generalized overlap), but again significantly different from 1.00 (chi-square = 724.09, $P < 0.001$). Thus, this burying beetle guild is comprised of species that may compete, but at least some species exhibit significant niche segregation.

Table 6. Niche breadths of seven species of burying beetles occurring in Oklahoma. Data are restricted to that for sites within counties known to be inhabited by N. americanus. All species exhibited highly significant habitat selectivity (i.e., all niche breadth values were significantly less than their theoretical maximum of 1.00; $P < 0.001$).

	ABB	CARO	MARG	ORBI	PUST	SAYI	TOME
Niche Breadth	0.78	0.21	0.36	0.71	0.53	0.60	0.89
Chi-square value	89.17	57.04	39.24	1132.99	25.07	43.84	99.31

Table 7. Pairwise niche overlap and segregation among four common species captured at sites within counties known to be inhabited by N. americanus. Values reported are overlap measures (row species on column species) which range from 1.00, complete overlap (no segregation) to 0.00 no overlap (complete segregation).

Species	ABB	ORBI	SAYI	TOME
ABB	----	0.505	0.235	0.844
ORBI	0.323	----	0.290	0.723
SAYI	0.131	0.535	----	0.687
TOME	0.469	0.597	0.475	----

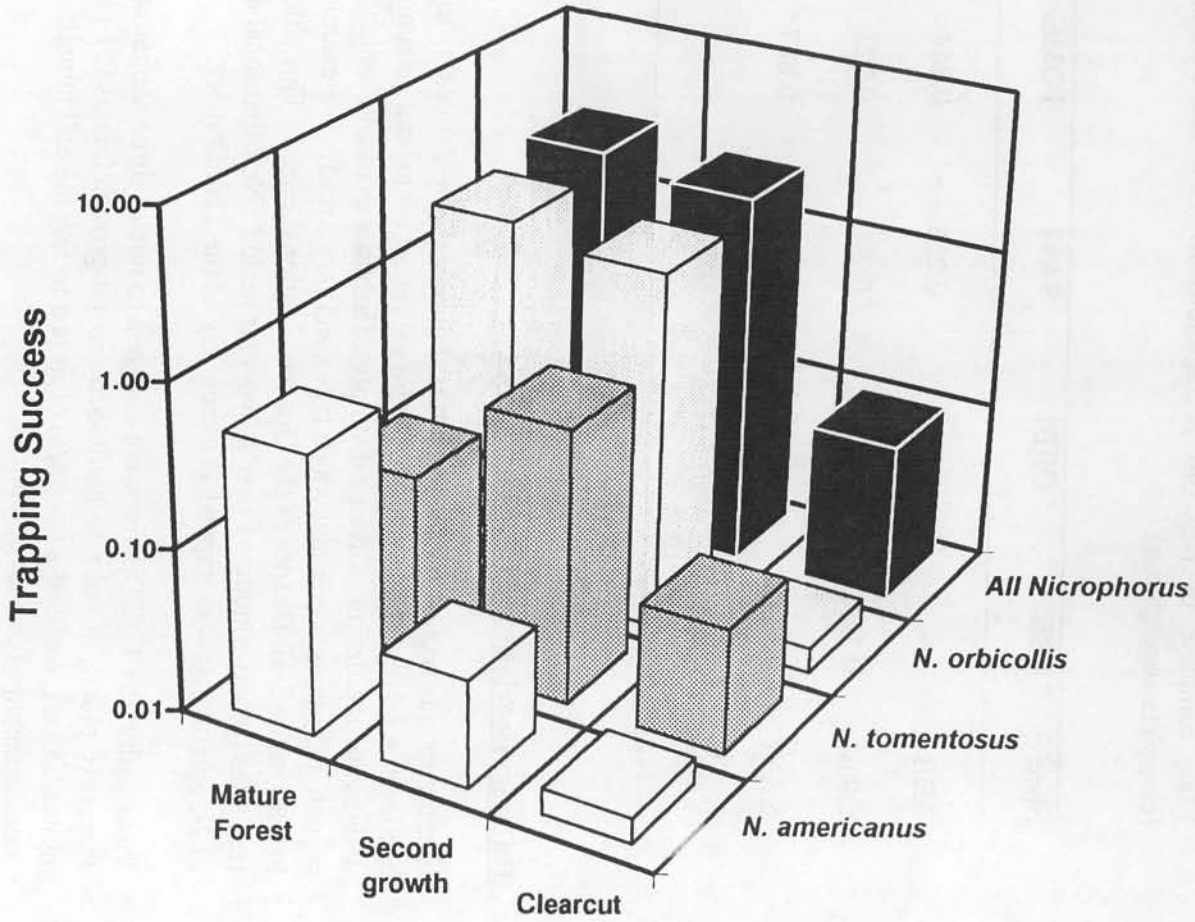
Habitat Associations at the Local Scale

In the previous section we analyzed distributions of populations or sub-populations of beetles at a relatively coarse scale. Here we report on habitat selection of individuals within one population. Given a diversity of habitats available, which types of habitats would individuals prefer and which types will they avoid? We examined this question for beetles in the Tiak District of the Ouachita National Forest. Our studies here included 20 trap lines (plots) conducted over a two-year period. No other local area within the range of N. americanus was trapped as intensively as the Tiak District.

Plots within the Tiak District were assigned to one of three habitat categories: mature forests (6 plots), mixed pine/hardwood second-growth forests (11 plots) and clearcuts (3 plots). Again, we used a goodness of fit test to test the null hypothesis that beetles were indiscriminant of habitat conditions.

N. orbicollis was by far the most common species encountered in the Tiak District (698 individuals over 435 functional trapnights), followed by N. tomentosus (132 individuals) and N. americanus (81 individuals). The distributions of these species, however, was not random with respect to habitat conditions. That is, all three species exhibited highly significant avoidance of clearcuts (Figure 2). In fact, N. americanus was the most specialized of the three common species encountered in the Tiak District (niche breadth =

Figure 2. Habitat Selection in the Tiak District, Ouachita National Forest



0.53, 0.80 and 0.84 for N. americanus, N. tomentosus and N. orbicollis, respectively; $P < 0.001$). N. americanus exhibited a strong preference for mature forests (Compartment 33), while N. tomentosus and N. orbicollis exhibited preferences for mature forests and second-growth forests over clearcuts.

Thus, when given a diversity of habitats, N. americanus exhibited a very strong selectivity for forested sites with deep soils and only modest to low density of undergrowth. These results are similar to those for analyses conducted at the landscape level, and are entirely consistent with the results the breeding experiments reported on in Section V. Therefore, certain forestry practices which promote these conditions (mature forests with deep soils and low to modest shrub cover) may be highly beneficial to this endangered species, while clearcutting, heavy thinning, soil erosion and compaction, and certain types of burning may jeopardize its populations.

III. CAPTIVE REARING OF BROODS.

1) Methods:

During September, 1992, 15 newly eclosed adults were captured in the field and then returned to the laboratory at the University of Oklahoma. After storage at winter conditions, the beetles were placed in laboratory environments simulating summer conditions to stimulate breeding. After breeding, adults were sent to A. Kozol at Boston University for genetic analyses (see additional methodology described in Report on Captive Breeding Studies, Appendix).

Brood chambers consisted of large plastic buckets which were half-filled with a mixture of potting soil and soil taken from the vicinity of capture. A pair of beetles was placed in each chamber with the carcass of a juvenile rat. The chambers were maintained under constant temperature and humidity. The progress of the beetles in burying the carrion and laying eggs was checked daily, as was larval development.

2) Summary of Results:

The results of these studies are described in detail in the attached report included as an appendix to this report. In summary, ten of eleven pairs of American burying beetles successfully reared at least one brood and five of eight pairs raised two broods. A total of 139 young was produced from these pairs. Four pairs of captive-bred adults were established on carcasses as well. Two of these pairs were successful in producing young. These pairs successfully reared young without being held in "winter-like" conditions. These results indicate that enough beetles can be raised in captivity for the purpose of establishing new populations.

IV. COLLECTION OF BEETLES FOR GENETIC COMPARISON BETWEEN POPULATIONS.

1) Methods:

Adults used in breeding experiments and trap losses from field studies were frozen to preserve the specimens prior to genetic analyses to be conducted at Boston University.

2) Results:

Mitochondrial DNA analyses have been completed and we do not expect additional specimens will be required. A. Kozol has submitted the final report of these studies to M. Amaral, USFWS.

V. BREEDING, HABITAT, AND INTERSPECIFIC COMPETITION.

Effects of Habitat on Breeding Success

1) Methods:

During 2-7 June 1994, 49 pairs of American burying beetles were captured for this study using baited pit-fall traps on the Cherokee Wildlife Management Area (Cherokee County) and Camp Gruber (Muskogee County). All American burying beetles captured were marked in two ways. Beetles were permanently marked by cutting a 3-mm triangular notch in the posterior portion of their right elytron. They also were marked individually with numbered bee tags affixed with gel super glue to the anterio-central portion of each beetle's right elytron.

Twenty-two 90 g rat carcasses were placed in grassland habitat and 21carcasses were placed in upland forest habitat on the Cherokee Wildlife Management Area. Dental floss was tied to each carcass so it could be located after burial. In the grassland, carcasses were placed on top of grass litter between vegetation clumps. In the forest, carcasses were placed on top of leaf litter away from undergrowth. In this way, the dental floss was less likely to become entangled in the surrounding vegetation, and microhabitat was controlled as much as possible within and between habitats. All carcasses were placed a minimum of two meters apart .

The grassland site was typical of old-field habitat (Küchler 1964). Common grasses at this site included Andropogon virginicus, Aristida sp., Sporobolus sp., and several species of Panicum. The upland, oak-hickory forest was dominated by Quercus stellata, Q. marilandica, Carya texana, and Ulmus alata.

A pair of marked beetles was placed on each carcass and the carcass was then covered with a 26 × 20 × 15 cm plastic tub. The tag number and size of each beetle (measured as pronotum width) were recorded at this time. The following day, the condition of each carcass (whether buried or not) was recorded. If no burying activity was observed, a second, marked pair was placed on the carcass.

Ten days after the final pair was placed on a carcass, it was carefully dug up. The presence and age of larva were recorded at this time. The number of larva was noted when larva had reached the third instar stage. Parents were captured if possible and their tag number recorded. In addition, size of parents (if they were different from original pair), depth carcass was buried (measured from the bottom of brood chamber to soil surface), and the numbers of fly larva on the carcass were recorded. The carcass was returned to its brood chamber and covered with leaves, dirt and a small, flat rock. Carcasses were checked daily until all data were recorded or the young dispersed into the soil.

2) Results:

Of the twenty-seven pairs of N. americanus placed on carcasses in the grassland, only 15 (56%) were successful in burying the carcass and rearing any young (Figure 3, Table 8). In contrast, 21 of 22 pairs (95%) placed on carcasses in the forested site were successful. Accordingly, the mean number of young raised per carcass placed in the grassland site was only 9.79, whereas 14.77 young were raised per carcass placed in the forested site. This difference in breeding success was statistically significant ($t = 2.18$, $P < 0.05$).

Carcasses tended to be buried deeper in the soil in grassland sites ($t = 3.61$, $P < 0.01$), whereas carcasses in forested sites were buried closer to the surface, just below the litter, and tended to have more fly larvae ($t = 2.09$, $P < 0.05$). Size of parents and number of young produced per successfully buried and initiated carcass was not significantly different among sites.

In summary, breeding success of the endangered American burying beetle was demonstrably lower in grassland versus forested sites (Figure 3). In fact, breeding success (number of young per available carcass) in grassland sites was only 66% of that for forested sites. This difference was primarily due to an apparent difficulty in securing and burying carcasses in grassland sites. For those carcasses that were successfully buried by N. americanus, site characteristics did not seem to influence subsequent breeding. We hypothesize that difficulty in securing carcasses in grasslands may result from the near absence of a litter layer and tendency for grassland soils to be more compact than those in forested sites. Grassland soils may thus be deemed less suitable for breeding or, because it takes longer to bury carcasses in grasslands, N. americanus may be more susceptible to competition from other burying beetles and from vertebrate scavengers.

Figure 3. Effects of Habitat on Breeding Success

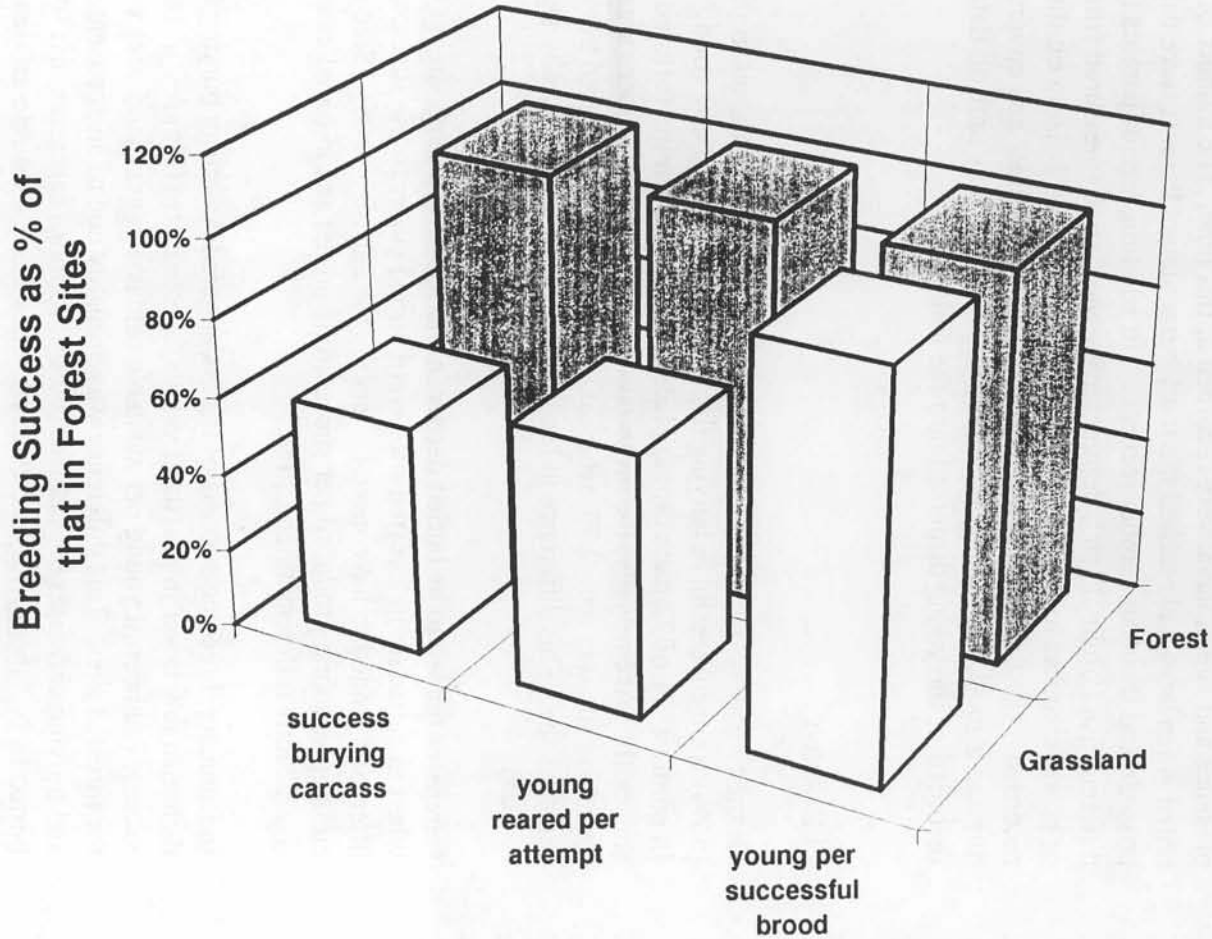


Table 8. Effects of habitat on breeding success of the American burying beetle. Successful breeding attempts are listed in bold. Depth buried is exclusive of litter. Female and male size refer to pronotum width. Age of young equals that taken 10 days after the the parents were placed on the carcass.

Table 8-a

N. americanus on carcasses in grassland sites in Cherokee County.

Carcass #	Depth buried (cm)	Female size (mm)	Male size (mm)	Number of young	Age of young	Number of fly larvae
G1	Buried; could not find	8.9	9.3	-	-	-
G2 (first attempt)	-	10.3	8.7	0	-	-
G2 (second attempt)	**11.3**	**-**	**-**	**9**	**4**	**15**
G3	**6.8**	**9.7**	**8.8**	**16**	**4**	**3**
G4	**13.1**	**8.5**	**9.2**	**22**	**4**	**0**
G5	**10.6**	**9.8**	**10.7**	**23**	**4**	**0**
G6 (first attempt)	-	10.1	10.3	0	-	-
G6 (second attempt)	9.8	-	-	0	-	25
G7 (first attempt)	-	11.3	9.3	0	-	-
G7 (second attempt)	**8.2**	**9.2**	**8.5**	**11**	**4**	**5**
G8 (first attempt)	-	9.1	9	0	-	-
G8 (second attempt)	**15.2**	**10.5**	**9.5**	**18**	**4**	**0**
G9	**9.4**	**10.8**	**10.1**	**15**	**1**	**0**
G10 (first attempt)	-	8.6	8.4	0	-	-
G10 (second attempt)	10.4	-	-	0	-	50
G11	Buried; could not find	9	8.8	-	-	-
G12	**13.1**	**8.5**	**8.7**	**16**	**4**	**0**
G13	**12.1**	**10.1**	**11.1**	**20**	**4**	**0**
G14	**11.6**	**9.5**	**10.7**	**11**	**4**	**5**
G15	**10.3**	**11.6**	**6.9**	**19**	**4**	**0**
G16	**12**	**9.5**	**8.5**	**19**	**4**	**6**
G17	Buried; could not find	10.1	9.2	-	-	-
G18	**6.3**	**10.8**	**9.9**	**10**	**4**	**25**
G19	**6**	**11.2**	**9.3**	**12**	**4**	**4**
G20	Never buried, No success	11.5	9.6	0	-	-
G21	**10.5**	**8.3**	**9.6**	**14**	**4**	**8**
G22	Never buried, No success	10.1	10.5	0	-	-

Table 8-a (continued)
Summary for Breeding in Grassland Habitat:

All Attempts						
mean	10.39	9.88	9.36	9.79	3.80	8.59
standard deviation	2.51	1.00	0.93	8.49	0.77	13.45
N	17	24	24	24	15	17
Successful Breeders						
mean	10.43	9.86	9.39	15.67	3.80	4.73
standard deviation	2.68	1.03	1.10	4.45	0.77	7.01
N	15	14	14	15	15	15

Table 8-b

N. americanus on carcasses in forested sites, Cherokee County.

Carcass #	Depth buried (cm)	Female size (mm)	Male size (mm)	Number of young	Age of young	Number of fly larvae
H1	**7.5**	**9.2**	**10.9**	**14**	**4**	**0**
H2	**6**	**9.8**	**8.2**	**3**	**4**	**100**
H3 (first attempt)	-	10.1	9.2	0	-	-
H3 (second attempt)	**7.8**	**10.6**	**9.4**	**16**	**4**	**0**
H4	**7.5**	**10.4**	**9.6**	**10**	**4**	**80**
H5	**9.7**	**11.9**	**10.6**	**21**	**2**	**0**
H6	**6.1**	**8.2**	**9.5**	**7**	**4**	**100**
H7	**6.6**	**9.6**	**8.6**	**6**	**4**	**30**
H8A	**7.5**	**7.3**	**11.4**	**8**	**-1**	**0**
H8B	**5.8**	**10.4**	**9.4**	**23**	**4**	**0**
H9	**6.6**	**9.6**	**9.8**	**15**	**4**	**1**
H10	**7.4**	**10.6**	**8.8**	**17**	**4**	**0**
H11	**6.4**	**10.1**	**9.8**	**13**	**4**	**70**
H12	**7.3**	**8.3**	**11.3**	**9**	**4**	**200**
H13	**8.5**	**10.5**	**8.8**	**15**	**4**	**1**
H14	**9.1**	**9.8**	**9**	**19**	**3**	**5**
H15	**11.2**	**9.9**	**9.8**	**22**	**4**	**0**
H16	**6.5**	**9**	**7.9**	**19**	**3**	**23**
H17	**6**	**7.9**	**8.6**	**23**	**4**	**100**
H18	**10.3**	**10.6**	**11.2**	**21**	**4**	**7**
H19	**7.1**	**10.9**	**9.7**	**19**	**4**	**0**
H20	**12.4**	**10.4**	**10.5**	**25**	**4**	**0**
Summary of Breeding in Forest Habitats:						
All Attempts						
mean	7.78	9.78	9.64	14.77	3.57	34.14
standard deviation	1.81	1.09	1.00	7.00	1.16	54.00
N	21	22	22	22	21	21
Successful Breeders						
mean	7.78	9.76	9.66	15.48	3.57	34.14
standard deviation	1.81	1.12	1.02	6.33	1.16	54.00
N	21	21	21	21	21	21

Table 8-c

Summary of breeding success of N. americanus in different habitats.

(note: mean values for young reared per attempt excludes carcasses buried, but not found. If we assume that these three carcasses were not successfully used for breeding by N. americanus, than mean number of young per attempt in grasslands = 8.70 (SD = 8.58, N = 27).

Habitat	Attempts	Success	Success rate	Young/attempt	Young/brood
Grassland	**27**	**15**	**56%**	**9.79**	**15.67**
Forest	**22**	**21**	**95%**	**14.77**	**15.48**
			t-value	**-2.18**	0.11
			P	< 0.05	> 0.90

Results of t-tests (grassland minus forest	Depth buried (cm)	Female size (mm)	Male size (mm)	# of young per carcass	Age of young	Number of fly larvae
all carcasses	**3.61**	0.32	-0.97	**-2.18**	0.71	**-2.09**

# of young per successful brood
0.11

Competition Studies:

1) Methods:

Interference Competition Experiments:

All competition experiments were conducted in forested sites, i.e., those apparently preferred by both N. orbicollis and N. americanus. Two treatments of this field study (Treatments 1 and 2) were located in the common grounds of Cherokee Wildlife Management Area (Cherokee County) and Camp Gruber (Muskogee County). The third treatment (Treatment 3) was located in the Tiak District of the Ouachita National Forest. In Treatment 1, a pair of N. americanus was placed on each of 21 carcasses. In Treatment 2, a pair of N. americanus and a pair of N. orbicollis were placed together on each of the remaining 20 carcasses, whereas Treatment 3 initiated N. orbicollis alone on 20 carcasses.

For Treatments 1 and 2, 41 pairs of the American burying beetle, Nicrophorus americanus, and 20 pairs of N. orbicollis were captured using baited pit-fall traps (Treatments 1 and 2 conducted during 2-7 June 1994). Forty-one 90g rat carcasses were placed in an upland forest habitat on the Cherokee Wildlife Management Area. These are relatively large carcasses within the optimal range for N. americanus (50 to 250 g), but beyond that for N. orbicollis. Dental floss was tied to each carcass so that it could be located after burial. Carcasses were placed on top of leaf litter away from undergrowth and at a minimum of two m apart. The carcasses were then covered with a transparent plastic tub (27 x 40 x 16 cm). Four to eight carcasses were observed per night, depending on the number of beetles caught that day. Observations on the degree of burial were recorded for approximately two hours per night at 15 to 30 minute intervals. The condition of each carcass was recorded the following day. Carcasses were exhumed ten days after burial. The species which successfully reproduced was recorded, along with the number of beetle larvae (larvae were counted once in the third instar stage), number of fly larvae, and depth of burial (measured from the bottom of the brood chamber to soil surface).

During 24-25 June 1994, 20 pairs of N. orbicollis were captured using baited pit-fall traps in the Tiak District of the Ouachita National Forest, McCurtain County. Twenty 90g rat carcasses were placed in a forest habitat above leaf litter and pine needles. Carcasses were monitored as for those in Treatments 1 and 2.

Exploitative Competition Experiments: Natural Settlement Studies

During 24-25 June 1994, thirty-two 50g and thirty-two 90g rat carcasses were placed at 16 forested sites in the Tiak District of the Ouachita National Forest, McCurtain County. Sites varied from one to 8 km apart. Each day, two carcasses, one of each size, were placed at each site 20m apart. All carcasses were set out by 1800 CST. One-half of the carcasses, sites 1-8 were observed at 45 minute intervals beginning at 2000 CST and ending at approximately 2450 CST. During these observations, the condition of the carcass was recorded, as well as the presence of any burying beetles and other insects and spiders. The following day, the condition of each carcass at all 16 sites was recorded. All buried carcasses were exhumed after ten days to determine which species reproduced, the number of beetle larvae, number of fly larvae, and depth of burial.

Observations during natural settlement studies were conducted on sites 1-8. Each night, a total of 16 carcasses was observed, one 50g and one 90g per site. Table 10-b shows those carcasses buried and those where beetles were seen. "Species present" refers to the species observed on the carcass once buried. If the dental floss and part of the carcass were found under ground with no evidence of beetles, it is referred to as "not found". It should be noted that the 50 g carcass at site 3 was not buried, but beetles were observed on the carcass the night before. "Set-up time" refers to the time in which carcasses were set out and "observations began" refers to the time when the first observation was made after set-up. After the first observation, observations were made at approximately 45 minute intervals.

2) Results:

Of the 21 large (90 g) carcasses initiated with just N. americanus, all but one were successfully buried by this species (Table 9-b, Figures 4 and 5). When both N. americanus and N. orbicollis were initiated on 21 large carcasses, N. americanus again secured all but one carcass (Table 9-a, Figures 4 and 5). Moreover, of the 20 large carcasses initiated with just N. orbicollis, nine were taken-over by free-ranging N. americanus, while none were secured by N. orbicollis (Table 9-c, Figures 4 and 5).

It appears that N. orbicollis had difficulty burying these relatively large carcasses, thus exposing it to takeover by the larger species, N. americanus. When carcasses were initiated with N. orbicollis, it failed to bury any carcass within one day (Table 10). Again, these large carcasses are probably beyond the optimal size for N. orbicollis, but well within the optimal range (50 to 250 g) for N. americanus.

Figure 4. Number of large (90 g) carcasses buried in forested sites by N. americanus and N. orbicollis. Carcasses initiated with N. orbicollis were located in an area with high density of N. americanus.

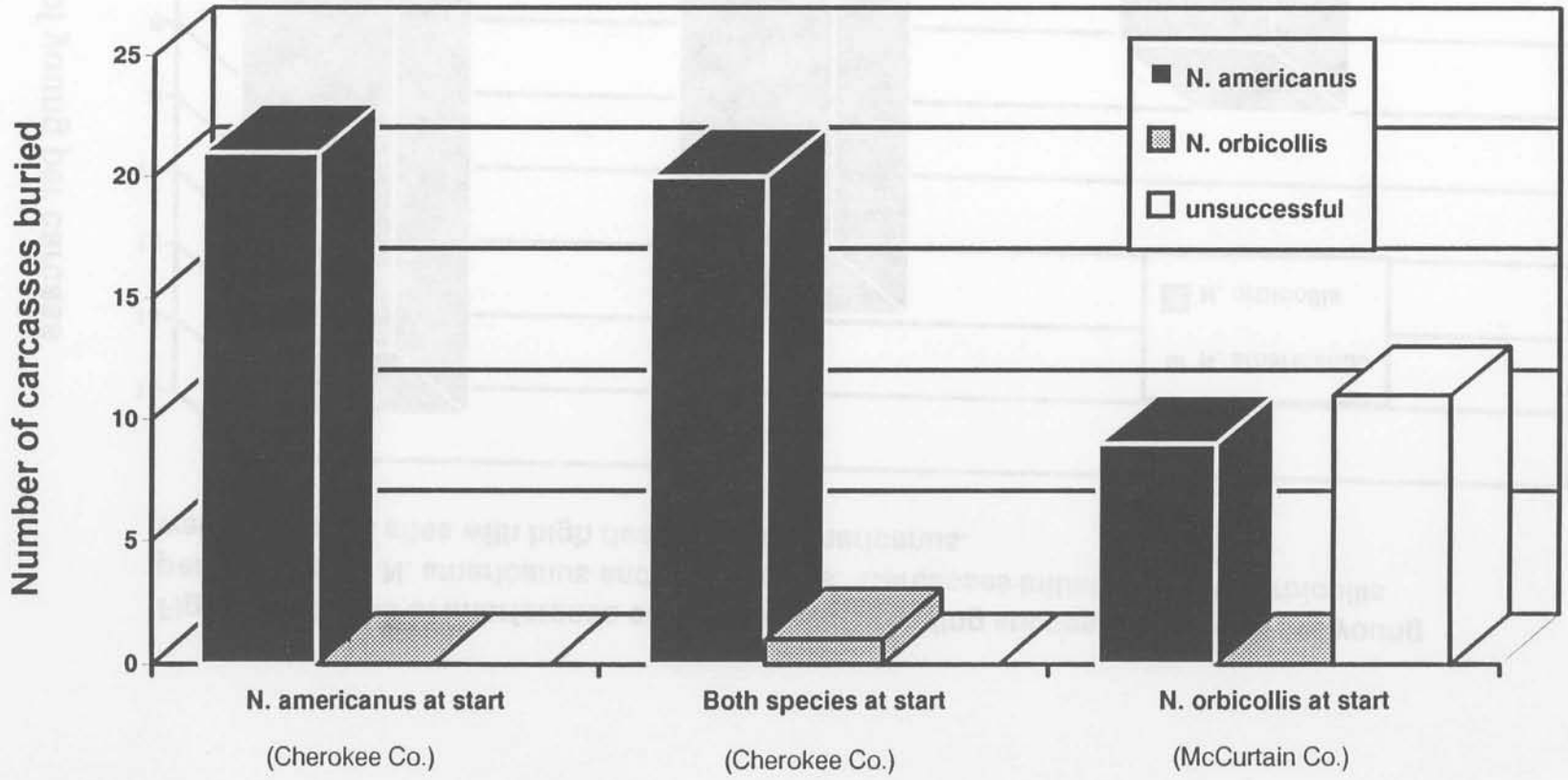


Figure 5. Effects of interference competition on breeding success (as number of young per carcass) of N. americanus and N. orbicollis. Carcasses initiated with N. orbicollis were located in sites with high desnity of N. americanus.

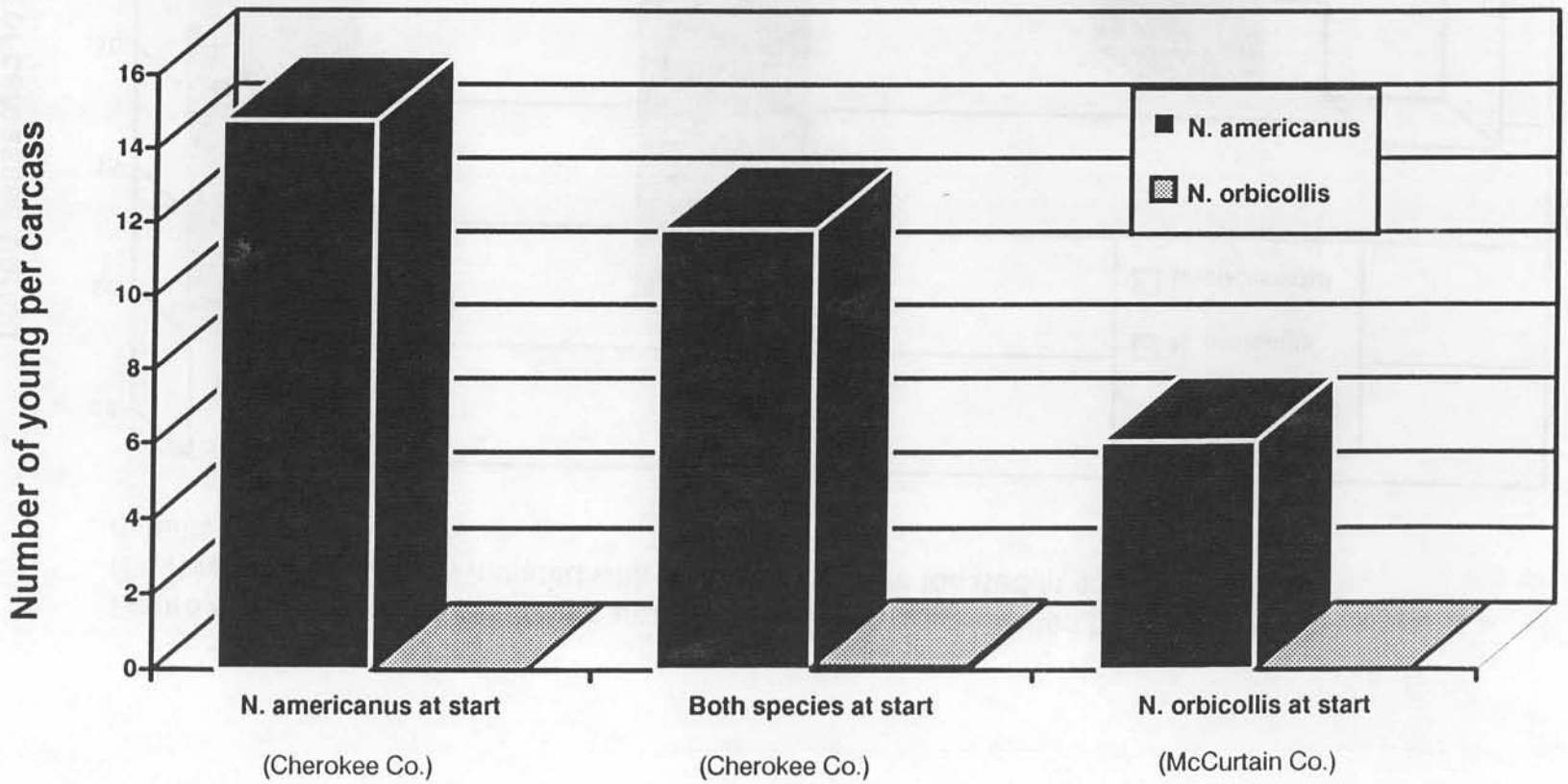


Table 9. Effects of interspecific competition on the breeding success of the American burying beetle, Nicrophorus americanus. Included are the results of the experiments in which N. americanus and N. orbicollis were placed together on carcasses (Cherokee Co when N. americanus (Cherokee Co.) was alone and when N. orbicollis (McCurtain Co.) was alone.

Table 9-a: Carcasses initiated with N. americanus and N. orbicollis : Cherokee County

Carcass #	Species present at end of experiment	Depth buried (cm)	# Young N. americanus	# Fly larvae	% of carcass under leaf litter after 90 minutes
C1	*N. americanus*	7.40	20	0	50
C2	*N. americanus*	8.70	16	15	0
C3	*N. americanus*	9.95	14	20	0
C4	*N. americanus*	7.10	12	0	0
C5	*N. americanus*	4.80	4	0	0
C6	*N. americanus*	4.60	17	0	0
C7	***N. orbicollis***	**3.30**	**0**	**50**	**0**
C8	*N. americanus*	8.10	15	0	0
C9	*N. americanus*	6.90	10	0	0
C10	*N. americanus*	11.00	0	100	25
C11	*N. americanus*	12.50	13	0	0
C12	*N. americanus*	11.90	16	10	100
C13	*N. americanus*	9.20	0	0	100
C14	*N. americanus*	9.10	16	15	0
C15	*N. americanus*	12.50	16	0	90
C16	*N. americanus*	8.30	7	0	0
C17	*N. americanus*	5.70	10	0	50
C18	*N. americanus*	8.90	15	15	0
C19	*N. americanus*	10.30	24	3	0
C20	*N. americanus*	9.50	15	0	0
C21	*N. americanus*	6.40	8	0	0

Competition - Initiated with N. americanus and N. orbicollis: Summary table

I. Buried by N. americanus

	Depth buried (cm) by ABB	# Young per carcass	# Fly larvae	% of carcass under leaf litter after 90 min.
Mean	8.64	11.81	8.90	20.75
S. D.	2.34	6.61	22.48	36.36
N	20	21	20	20

N. orbicollis (19 young on carcass C7)

Mean	3.30	0.90	50.00	0
S. D.	-----	-----	-----	-----
N	1	1	1	1

Table 9-b: Carcasses initiated with N. americanus only (2nd refers to a second attempt)

Carcass #	Species present at end of experiment	Depth buried (cm)	# Young	# Fly larvae	% of carcass under leaf litter after 90 min.
H1	*N. americanus*	7.50	14	0	50
H2	*N. americanus*	6.00	3	100	0
H3(1st)	-	-	0	-	0
H3 (2nd)	*N. americanus*	7.80	16	0	0
H4	*N. americanus*	7.50	10	80	50
H5	*N. americanus*	9.70	21	0	50
H6	*N. americanus*	6.10	7	100	25
H7	*N. americanus*	6.60	6	30	25
H8A	*N. americanus*	7.50	8	0	25
H8B	*N. americanus*	5.80	23	0	not observed
H9	*N. americanus*	6.60	15	1	0
H10	*N. americanus*	7.40	17	0	0
H11	*N. americanus*	6.40	13	70	0
H12	*N. americanus*	7.30	9	200	33
H13	*N. americanus*	8.50	15	1	90
H14	*N. americanus*	9.10	19	5	0
H15	*N. americanus*	11.20	22	0	0
H16	*N. americanus*	6.50	19	23	25
H17	*N. americanus*	6.00	23	100	0
H18	*N. americanus*	10.30	21	7	0
H19	*N. americanus*	7.10	19	0	75
H20	*N. americanus*	12.40	25	0	33

Carcasses initiated with N. americanus only: Summary table

All were buried by N. americanus

	Depth buried (cm)	# Young per carcass	# Fly larvae	% of carcass under leaf litter after 90 min.
Mean	7.78	14.77	34.14	22.90
S. D.	1.81	7.00	54.00	27.28
N	21	22	21	21

t-tests of the effects of interference competition on breeding success of N. americanus.

	number of young per carcass	
	N. americanus at start	**both species at start**
Mean	14.77	11.81
S. D.	7.00	6.61
N	22	21

t-value = 1.43

not significant

Table 9-c: Carcasses initiated with N. orbicollis only: McCurtain County
These carcasses were not observed overnight, remarks are included

Carcass #	Species present at end of experiment	Depth buried (cm)	# Young	# Fly larvae	Remarks
T1	*N. americanus*	13.00	12	0	buried 1st night
T2	*N. americanus*	19.50	17	40	buried 1st night
T3	*N. americanus*	12.00	14	0	50% buried 1st night
T4	*N. americanus*	9.50	6	15	under needles 1st and 2nd nights
T5	unsuccessful	0.00	0	-----	50% under needles
T6	unsuccessful	0.00	0	-----	75% under needles
T7	unsuccessful	0.00	0	-----	25% buried, N. americanus seen
T8	unsuccessful	0.00	0	-----	under needles
T9	unsuccessful	0.00	0	-----	25% under needles, part taken by scave
T10	unsuccessful	0.00	0	-----	not buried
T11	*N. americanus*	11.00	12	0	buried 2nd night
T12	unsuccessful	0.00	0	-----	not found
T13	unsuccessful	0.00	0	-----	not buried
T14	unsuccessful	0.00	0	-----	not buried
T15	*N. americanus*	15.00	20	0	not buried 1st night
T16	*N. americanus*	13.00	11	50	not buried 1st night
T17	*N. americanus*	11.00	11	15	not buried 1st night
T18	unsuccessful	0.00	0	-----	not buried
T19	unsuccessful	0.00	0	-----	not buried
T20	*N. americanus*	7.50	18	4	not buried 1st night

Carcasses initiated with N. orbicollis only: Summary table

All buried carcasses found had N. americanus present

	Depth buried (cm)	# Young
Mean	5.58	6.05
S. D.	6.70	7.41
N	20	20

t-tests of the effects of explitative competition on breeding success of N. americanus.

	number of young per carcass	
	N. americanus at start	**N. orbicollis at start**
Mean	14.77	6.05
S. D.	7.00	7.41
N	22.00	20.00

t-value = **3.91**

$P < 0.001$

N. americanus, however, may also suffer from interference competition. When both species were placed on a carcass, breeding success of N. americanus was reduced from 14.77 to 11.81 young per carcass ($t = 1.43$, $0.10 < P < 0.20$; Table 9-b).

In contrast to the results of breeding experiments where one or both species were placed on a carcass, natural settlement of beetles on carcasses indicated that N. orbicollis dominates in exploitative competition (Figure 6, Table 10). N. orbicollis, which was eight times as abundant as N. americanus (Table 2, McCurtain Co.), secured seven carcasses placed in forested sites, while N. americanus secured just one. As a result, N. orbicollis reared 103 young, while N. americanus reared only seven (Table 10). The only carcass secured by N. americanus was one located in an area with the highest local density of N. americanus (Compartment 33 of the Tiak District, Ouachita National Forest).

These results are consistent with those of breeding experiments which also indicate that breeding success of N. americanus is substantially lower if N. orbicollis is first to locate a carcass (Figures 4 and 5; compare results for treatments with carcasses initiated with N. americanus versus those initiated with N. orbicollis). Breeding success of N. americanus was reduced from 14.77 young per carcass (carcasses initiated with just N. americanus) to only 6.05 young per available carcass when N. orbicollis was given first access to the carcass (Table 9-c, $t = 3.91$, $P < 0.001$).

Competition experiments indicated that the first individual to encounter a carcass was often not the one that eventually bury the carcass. During field experiments where both N. americanus and N. orbicollis were placed on a carcass, there were rarely any direct encounters between the two species. N. orbicollis usually simply disappeared in the leaf litter as N. americanus began preparing the carcass. When there was an encounter, it was brief. N. americanus either moved towards N. orbicollis and N. orbicollis quickly scurried off, or contact was made but no fighting was observed. N. americanus was clearly dominant in all encounters. It appeared that in some situations, N. americanus "allowed" N. orbicollis to remain on the carcass for a short time, with all beetles feeding on the carcass. Yet, N. orbicollis would disappear soon after this. In the one case where N. orbicollis did bury the carcass, N. americanus was observed leaving the enclosure soon after the beetles were placed on the carcass, and no activity was observed for the rest of the observation time. The carcass was found buried the next morning. There was one case in which it appeared N. orbicollis might win the carcasss, but never actually did. Soon after the pairs of beetles were placed on the carcass, the male N. americanus attempted to mount the female. This continued for the next hour, while N. orbicollis prepared and fed on the carcass. Once the pair of N. americanus commenced mating and moved onto the carcass, N. orbicollis left.

Observations conducted during natural settlement studies suggest that beetles are not arriving especially early in the evening, with the exception of site 4-90g. Of the

nine buried carcasses in sites 1-8, three of them lacked any evidence of beetle activity at the time of the last observation (between 12:00a.m. and 1:00a.m.). Yet once beetles arrive, carcass preparation and burial follow quickly, regardless of the presence of another beetle.

The preponderance of N. orbicollis over N. americanus on carcasses is consistent with the differences in local densities of these species. Only sites 2 and 3 were located near relatively high densities of N. americanus (Compartment 33). Of all carcasses set out for natural settlement, only site 2-50g was successfully buried by N. americanus. Outside of Compartment 33, N. orbicollis is in such high numbers, that it can successfully bury a carcass before N. americanus can arrive. On the other hand, competition experiments show that N. americanus can dominate in interference competition. This appears to have occurred at site 2-50g, where N. orbicollis was first observed on the carcass, but N. americanus was the species found to have successfully reproduced.

It is also important to note that most carcasses used in the natural settlement study were taken by vertebrate scavengers (primarily raccoons and opossums) or were not buried (Figure 6).

Taken together, breeding experiments and natural settlement studies indicate that N. americanus, the larger species, dominates in interference competition (especially for larger carcasses), whereas, N. orbicollis, the more abundant species, dominates in exploitative competition. Both species also may suffer substantially from competition and predation from vertebrate scavengers.

VI. ANALYSIS OF DATA AND ANNUAL REPORT

We have accomplished all of the tasks included in the project description. What follows are recommendations for management based on the information reported in this study.

Figure 6. Fates of 64 carcasses placed at 16 sites in the Tiak District of Ouachita National Forest, McCurtain County.

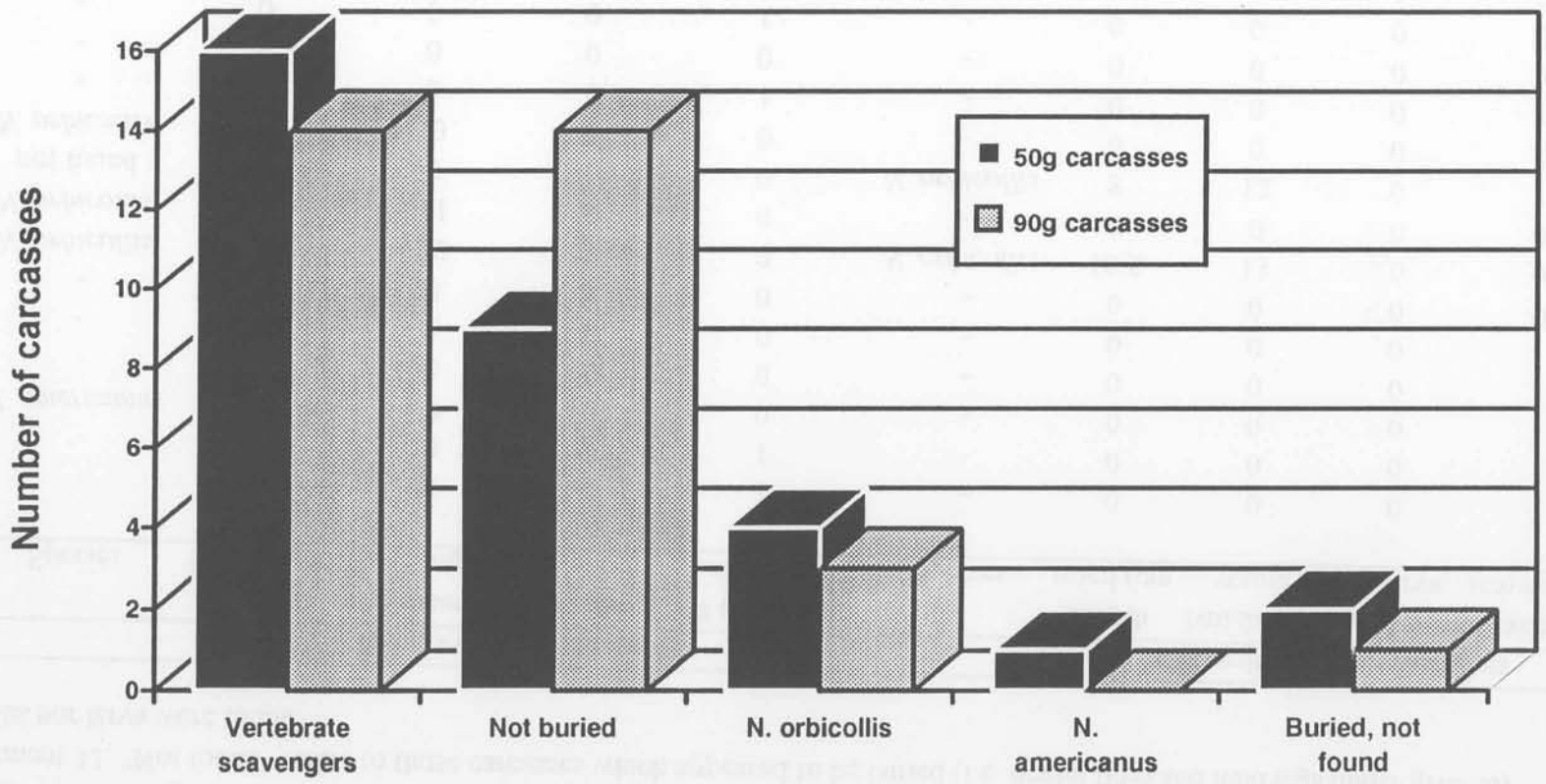


Table 10-a. Natural settlement of burying beetles on carcasses placed in forested sites of the Tiak District, Ouachita National Forest. Two 50 g and two 90 g carcasses were located at each of 16 sites for a total of 64 carcasses. Sites 2 and 3 were located in Compartment 33. "Not found" refers to those carcasses which appeared to be buried (i.e. dental floss and hind legs under ground) but beetles nor larva were found.

	Results for 50 g carcasses					Results for 90 g carcasses				
Site	Species	Depth buried (cm)	Number of young	Number of fly larva	# taken by scavengers	Species	Depth uried (cm	Number of young	Number of fly larva	# taken by scavengers
1-a	-	0	0	0	1	-	0	0	0	1
1-b	-	0	0	0	1	-	0	0	0	1
2-a	*N. americanus*	9	7	0	0	-	0	0	0	1
2-b	-	0	0	0	0	-	0	0	0	1
3-a	-	0	0	0	0	-	0	0	0	1
3-b	-	0	0	0	0	-	0	0	0	0
4-a	*N. orbicollis*	8.5	22	0	0	*N. orbicollis*	10.5	13	0	0
4-b	*N. orbicollis*	20.7	11	0	0	-	0	0	0	0
5-a	not found	0	0	0	0	*N. orbicollis*	8	12	0	1
5-b	*N. orbicollis*	5	13	0	0	-	0	0	0	0
6-a	-	0	0	0	1	-	0	0	0	1
6-b	-	0	0	0	0	-	0	0	0	0
7-a	-	0	0	0	1	-	0	0	0	1
7-b	-	0	0	0	1	-	0	0	0	0
8-a	*N. orbicollis*	7.5	5	35	1	not found	0	0	0	1
8-b	-	0	0	0	0	-	0	0	0	0
9-a	-	0	0	0	1	-	0	0	0	0
9-b	-	0	0	0	0	-	0	0	0	0
10-a	-	0	0	0	0	-	0	0	0	1
10-b	-	0	0	0	0	-	0	0	0	1
11-a	-	0	0	0	1	-	0	0	0	0

11-b	-	0	0	0	1	-	0	0	0	0
12-a	not found	0	0	0	1	-	0	0	0	1
12-b	-	0	0	0	0	-	0	0	0	0
13-a	-	0	0	0	0	-	0	0	0	0
13-b	-	0	0	0	1	-	0	0	0	0
14-a	-	0	0	0	1	*N. orbicollis*	9.5	27	3	0
14-b	-	0	0	0	1	-	0	0	0	0
15-a	-	0	0	0	1	-	0	0	0	1
15-b	-	0	0	0	1	-	0	0	0	1
16-a	-	0	0	0	1	-	0	0	0	0
16-b	-	0	0	0	0	-	0	0	0	0

Summary (all carcasses, 50 g and 90 g):

Carcasses buried by N. americanus:

	Depth buried (cm)	Number of young	Number of fly larva
N = 1	9	7	0

Carcasses buried by N. orbicollis

	Depth buried (cm)	Number of young	Number of fly larva
Total		103	38
Mean	9.96	14.71	5.43
SD	5.04	7.36	13.09
N	7	7	7

Table 10-b: Results of observations on Natural Settlement sites 1-8 conducted in the Tiak District of McCurtain County (two 90g and two 50g at each site). Table includes those carcasses buried and those where beetles were seen. All beetles observed were N. orbicollis.

Site #	Carcass mass (g)	Species present	Set-up time	bservation began	1st beetle observed	2 beetles present	1st evidence of burial
2	50	N. americanus	6:33p	8:22p	11:40p	-	12:30a
3	50	-	6:10p	8:06p	12:12a	-	-
4	50	N. orbicollis	6:22p	8:11p	-	-	-
4	50	N. orbicollis	6:15p	8:03p	-	-	-
4	90	N. orbicollis	6:22p	8:11p	8:11p	10:44p	9:01p
5	50	not found	6:15p	8:07p	9:49p	-	-
5	50	N. orbicollis	6:00p	8:00p	12:01a	-	12:54a
5	90	N. orbicollis	6:00p	8:00p	9:28p	12:01a	12:54a
8	50	N. orbicollis	5:42p	7:50p	-	-	10:57p
8	90	not found	5:35p	7:45p	10:05p	-	-

VII. RECOMMENDATIONS FOR MANAGEMENT AND RECOVERY

Investigator's Summary:

Live-trapping, including 4,232 trapnights conducted at 179 sites across 20 counties, was conducted to study the endangered American burying beetle (Nicrophorus americanus) in Oklahoma. Out of a total 6,374 burying beetles captured, including seven species, only 3.2% were American burying beetles. N. americanus was detected in nine counties along the central- to south-eastern portion of the state (Figure 1). The highest population densities of N. americanus occurred in Muskogee County. The second highest densities of beetles were encountered in McCurtain County, but density here was less than 20% of that in Muskogee County.

All species encountered exhibited highly significant habitat selection at both the landscape level (i.e., across counties) and at the local scale (sites within McCurtain County). At the landscape level, N. americanus exhibited the second broadest habitat niche, but it still exhibited highly significant preference for forested sites with deep soils and moderate shrub cover. At the local scale (20 sites in the Tiak District of Ouachita National Forest, McCurtain County), all burying beetles, including N. americanus, exhibited a highly significant preference for forested sites and avoidance of clearcuts. Therefore, while N. americanus may exhibit a broad habitat niche in comparison to most (but not all) other burying beetles, it still exhibits strong associations for particular habitats.

Observed habitat associations of N. americanus likely reflect its breeding requirements. As Anderson (1982) suggested, because N. americanus buries relatively large carcasses, viable populations of this species may be dependent on habitats with relatively loose and deep soils with a substantial litter layer. In breeding experiments in the field we found that breeding success of N. americanus was 50% higher in forested habitats versus grasslands.

Field experiments also indicated that populations of burying beetles are strongly influenced by interspecific competition. N. americanus, the largest species, dominated in interference competition, whereas smaller but more abundant species dominated in exploitative competition.

During laboratory studies, we developed a successful protocol for rearing individuals from the western population of N. americanus. Ten of 11 pairs of this species successfully reared at least one brood in the lab, while five of eight pairs raised two broods. Finally, to assist genetic studies, adults used in breeding experiments and trap losses from field studies were frozen and forwarded to A. Kozol of Boston University.

In conclusion, the American burying beetle belongs to a guild of species that feeds and breeds on resources (carcasses) that are rare and unpredictable in time and space. Because it is larger than other members of this guild, N. americanus requires carcasses that are even larger and more rare. Because larger carcasses are more difficult to bury, optimal breeding habitats appear to be forests and possibly other sites with substantial litter and

relatively deep, loose soils. Also, because they search for rare, high energy resources, N. americanus apparently suffers from competition from other, more common burying beetles. Moreover, N. americanus suffers from competition and predation from vertebrate scavengers. As others have suggested, the recent and dramatic decline of N. americanus has apparently resulted from a suite of anthropogenic disturbances that reduced, fragmented and degraded their breeding habitats, reduced densities of their prey (large vertebrate carcasses) or enhanced populations of their competitors and predators.

Recommendations for Management and Conservation:

On the basis of the results of our studies, we offer the following recommendations for conservation and management of western populations of the American burying beetle.

1) Because the efficacy of the live-trapping protocol is well demonstrated, we recommend that these procedures be used in future surveys of western populations of the American burying beetle. Surveys are time- and cost-efficient and they should be conducted when proposed activities will likely alter the habitat, soil, competitors or predators.

2) Patterns in temporal variation in population densities of N. americanus indicate that surveys of western populations of this species should be restricted to the period from early June to late August (Figure 7). Beetles may be detected at other times, but absence of N. americanus in surveys conducted outside the recommended period is only questionable evidence that they do not occur at the focal site.

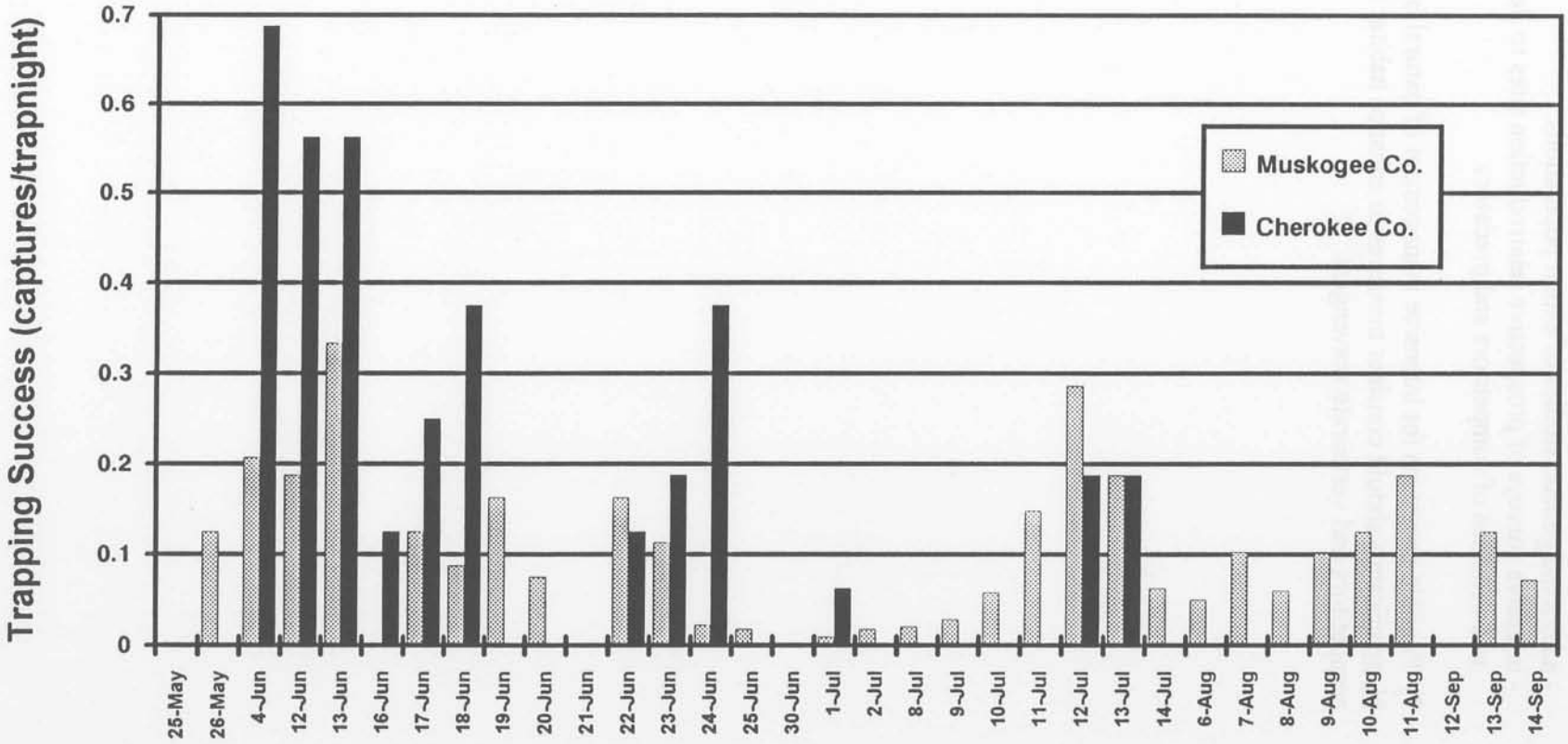
3) Additional studies on the ecology and breeding biology of N. americanus should be conducted. We recommend these include additional manipulative field studies on the influence of habitat on breeding success and the importance of interspecific competition from other burying beetles and competition and predation from vertebrate scavengers. In addition, the potential mutualistic and parasitic effects of mites in the laboratory and the field, and the optimal carcass size should be studied.

4) At sites known to be inhabited by N. americanus, substantial thinning, soil disturbance or compaction, clearcutting, burning during the activity and breeding season, and other activities that substantially alter the habitat or enhance populations of competitors or predators should be avoided.

5) Re-introduction programs for western populations of N. americanus should consider the habitat association of this species and its likely dependence on sites with soils that facilitate rapid burial of carcasses.

6) Prior to re-introductions of N. americanus into areas of its former range, we recommend the following:

Figure 7. Temporal variation of trapping success at two sites with high densities of N. americanus (1992).



- extensive surveys to discover possible, extant populations in the area.
- additional genetic studies of extant populations.
- intensive surveys of prospective reintroduction sites to determine suitability of habitat and densities of competitors and predators.

7) Possible programs for intensive management of natural or re-introduced populations of N. americanus should consider measures to enhance habitat suitability and control competitors and vertebrate scavengers.

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Date: 15 October 1994

Approved Oklahoma Department of Wildlife Conservation

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APPENDIX I

REPORT ON THE AMERICAN BURYING BEETLE CAPTIVE BREEDING PROGRAM AT THE UNIVERSITY OF OKLAHOMA

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Internal Report: Not to be cited.

I. Introduction

Populations of the American burying beetle (*Nicrophorus americanus*) have suffered a precipitous drop in numbers and geographic range in recent years. As a result, *N. americanus* was designated as an endangered species in July, 1988. In an effort to learn more about the reproductive biology of the species, we began a captive breeding program at the University of Oklahoma. Several goals were established: (1) Establish a protocol for breeding the beetle at the University of Oklahoma; (2) Provide replacements for wild-caught beetles used for genetic analysis; (3) Determine whether enough beetles could be raised in the laboratory for re-introduction programs and (4) Assess the viability of laboratory reared beetles for reintroduction programs.

II. Material and Methods

A. Handling of beetles

All beetles were captured using procedures outlined by Creighton et al. (1993). In 1991, three pairs were captured during June 4-6 in Latimer County, Oklahoma. On September 14, 1992, seven pairs plus an additional female were captured in Muskogee County, Oklahoma. Once in the laboratory, all beetles were kept in environmental chambers on the University of Oklahoma campus. The three pairs from Latimer County were returned to the University of Oklahoma and placed on carcasses on June 7, 1991. The beetles captured in Muskogee County were kept at 7° C and 10:14 light-dark cycle for seven weeks and at 21° C, 14:10 light-dark cycle for one week (eight weeks total) prior to being given carcasses. While breeding, all pairs were kept at 21° C and 14:10 light-dark. Approximately one week after young dispersed from the first carcass, each pair was placed in a new container and given a second carcass. Prior to being given a carcass and between breeding attempts, all pairs were kept in 5-gallon plastic buckets lined with moist

paper towels. Beetles were provided with small pieces (less than 5 grams) of chicken liver ad libitum. After the second breeding attempt, each pair was frozen and sent to Andrea Kozol at Boston University. The exceptions were several beetles that died prior to being frozen. These are currently being housed at the Oklahoma Biological Survey.

B. Preparation of breeding containers

Five gallon plastic buckets were used as breeding containers. Each bucket was filled approximately 3/4 full with top soil. If necessary, the soil was moistened with water before being added to the buckets. The soil should be moist enough so that it can clump together when balled but not so moist as to be muddy or too sticky. As the soil was added to the bucket, it was lightly packed by hand several times.

A single pair (male and female) of beetles was added to each bucket along with a dead white rat. Rats ranged in size from 39 to 163 grams. Moist paper towel was laid over the rat and the bucket was then placed in an environmental chamber and not disturbed for eight to ten days. After this time, each bucket was checked daily until the young dispersed into the soil.

C. Care of laboratory bred adults

The sex and pronotum width of each newly eclosed adult were recorded. The laboratory-bred beetles were kept in buckets, either with soil or moist paper towels. All beetles were provided ad libitum chicken liver. Only the young originating from the Muskogee County population were kept in captivity. These beetles were kept on one of two different substrates: soil or moist paper towels. This was done in an effort to see whether either substrate was more successful in keeping the beetles' phoretic mites alive. However, the containers with soil became infected with large numbers of a second, unidentified mite. This may have contributed to the death of many of the lab-reared beetles. These young were kept at 21° C, 14:10 for eight weeks (same amount of time their parents were kept in captivity before they were bred) and then attempts were made to breed the young. Except for the differences in temperature and light, these beetles were treated the same as their parents.

III. Results and Discussion

A. Laboratory breeding success

Ten of the eleven pairs of wild-caught beetles successfully reared at least one brood. Of the ten successful pairs, nine produced a brood on their first attempt. The tenth pair produced a brood on their second breeding attempt. Eight pairs were given a second carcass. Five of these pairs were successful. A total of fifteen broods was raised. This resulted in 139 adult young. A complete summary of breeding results is found in Table 1.

Pair number two was the only pair that did not successfully reproduce in the laboratory (Table 1). The pair removed the hair on the carcass but they failed to bury it. One possible explanation for their lack of success may be the condition of the male. When initially trapped, he already had extremely worn mandibles and only four legs. this was the only individual in this condition in all eleven pairs. Pair nine was unsuccessful on their first breeding attempt. This may have been due to the small size of their carcass (39.4 g). This pair did not attempt to prepare or bury this carcass. However, the pair immediately began work on the second carcass (97 g) on their second attempt.

We found no relationship between carcass size and number of young raised (Figure 1). This is counter to observations made by other researchers for the American burying beetle (Kozol et al. 1988) and for other species of burying beetles as well (Trumbo 1993; Creighton unpublished data). This may be due to either poor egg or larva survivorship in some of our broods. At this time, we do not know and more study is needed.

B. Release of young to minimize impact of adult removal

The young resulting from the Latimer County beetles were released back into the wild at the site of their parents' capture. Young originating from the Muskogee County beetles were kept in the laboratory for breeding purposes. In the months prior to their parents' capture, carcasses were placed out in the field in Muskogee County. A minimum of nine carcasses was buried by American burying beetles and these produced a minimum of 79 third instar young. This was done partially to compensate for any negative impact the removal of 15 adults used in the captive-breeding experiment might have on the wild population.

C. Use of Laboratory Population for re-introduction programs

Two of the four pairs of captive-bred beetles successfully produced young. Production of young, however, was very low with only two adults produced by the two successful pairs (Table 2).

The two pairs of beetles that did not produce young did prepare and partially bury their carcasses. However, both carcasses had extremely large numbers of an unidentified mite on them (i.e., many more mites than the two carcasses where young were produced). It is not clear whether the mites had any negative effect on the beetles' reproductive efforts. Survivorship of the young for the two successful pairs was low. What may have contributed to this fact is not clear.

It should be noted that these beetles were never kept in "winter-like" conditions (see methods for details). However, it appears that there were more than one generation of American burying beetles per summer (Creighton unpublished data) in the Oklahoma population. Therefore, Oklahoma beetles may not need to be "winterized" prior to becoming reproductively active.

Captive-bred beetles were kept on two different substrates. Those kept on soil became covered with a small, white mite. At the same time, the phoretic mites often seen on burying beetles disappeared from all beetles, whether the were kept in soil or paper towels. For this reason, re-introduction attempts may be most successful when a mixture of wild-caught and captive-bred individuals are used.

IV. Summary

Eleven pairs of American burying beetles were given carcasses in the laboratory. Ten pairs successfully raised at least one brood and five pairs raised two broods. A total of 139 young was produced from these pairs. Four pairs of captive-bred adults were established on carcasses as well. Two of these pairs were successful in producing young. These pairs successfully raised young without being held in "winter-like" conditions. These results indicate that enough beetles can be raised in captivity for the purpose of establishing new populations.

Table 1. Breeding success of captive pairs. Pronotum width refers to size of offspring.

Pair Number	Starting Date	Carcass Size	Number Third Instar Larvae	Number Adults	Sex Ratio (M:F)	Mean Width of Pronotum
1	6/7/1991	99.3 g	20	20	9:11	10.9 mm
1 (2nd brood)	7/2/1991	97.9 g	No Success			
2	6/7/1991	97.0 g	No Success			
3	6/7/1991	96.3 g	12	12	-	-
4	11/9/1992	163 g	9	8	5:3	10.9 mm
4 (2nd brood)	12/8/1992	108 g	11	11	6:5	11.7 mm
5	11/9/1992	132 g	18	15	3:12	11.1 mm
5 (2nd brood)	12/8/1992	86.4 g	21	9	3:6	11.0 mm
6[1]	11/9/1992	71.7 g	5	3	2:1	11.9 mm
7	11/9/1992	117.2 g	5	5	2:3	12.5 mm
7 (2nd brood)	12/8/1992	61 g	No Success			
8	11/9/1992	52.8 g	11	10	5:5	10.1 mm
8 (2nd brood)	12/8/1992	61 g	16	15	9:6	9.7 mm
9	11/9/1992	39.4 g	No Success			
9 (2nd brood)	12/1/1992	97 g	8	8	6:2	11.0 mm
10	11/9/1992	60 g	4	4	3:1	11.8 mm
10 (2nd brood)	12/8/1992	61 g	No Success			

Table 1. continued

Pair Number	Starting Date	Carcass Size	Number Third Instar Larvae	Number Adults	Sex Ratio (M:F)	Mean Width of Pronotum
11	11/9/1992	104.5 g	4	4	2:2	11.5 mm
11 (2nd brood)	12/8/1992	51.9 g	12	11	6:5	10.7 mm
TOTALS			160	139	63:64	

[1]Pair six consisted only of a single female. No second breeding attempt was made.

Table 2. Breeding success of captive-bred beetles. Source refers to brood from which the beetles originated. All beetles used were from second broods. Pronotum width refers to size of offspring.

Source (M,F)	Starting Date	Carcass Size	Number Third Instar Larvae	Number Adults	Sex Ratio (M:F)	Mean Width of Pronotum
1,2	3/7/1993	72 g	No Success			
8,6	3/7/1993	61 g	No Success			
2,1	3/9/1992	68 g	4	1	1 (Male)	11.61
1,2	3/9/1993	61 g	4	1	1 (Male)	11.45
TOTAL			8	2	2:0	

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APPENDIX II

SURVEY METHODS FOR THE AMERICAN BURYING BEETLE (NICROPHORUS AMERICANUS) IN OKLAHOMA AND ARKANSAS

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Introduction

The American burying beetle (*Nicrophorus americanus*) is the largest member of the genus *Nicrophorus* in North America. It ranges from 1 to 1.5 inches (25-45 mm) in length. Like most other burying beetles, the American burying beetle has four red-orange spots on its wing covers (elytra). It can be distinguished from other North American burying beetles by its larger size and its orange-red pronotum and frons (see Figure 1).

The disappearance of the American burying beetle from over 90 percent of its historic range underscores the need for consistent, reliable methods when surveys for the beetle are conducted (U.S. Fish and Wildlife Service, 1991). The methods outlined below have proven to be successful in capturing the American burying beetle. Following these methods should help to ensure the validity of survey results. Furthermore, data gathered using these methods will allow for easier comparison of results from different surveys.

Site Selection

American burying beetles are generalists, occurring in many different habitats. Therefore, surveys should be conducted in a broad range of habitats. In addition, individual beetles have been recorded moving over 4 miles (6.5 km) in only a few days. For this reason, there is no need to locate survey sites less than one-half mile (0.8 km) apart. If large areas are being surveyed, sites can be located as much as one mile (1.6 km) apart. Individual sites should be trapped for three nights.

Trapping Methods

Baited pitfall traps are the most effective method known for surveying for American burying beetles. At each site, eight pitfall traps are placed at 20-m intervals along a transect line (Figure 2).

Each pitfall trap consists of two, 24-oz. (0.7-L) plastic cups stacked together and buried in the ground so that the lip of the top cup is flush with the soil surface (Figure 3). A plastic dome should be placed over each trap to keep out rain. A 10 x 10 inch (25 x 25 cm) piece of wood (held above the pitfall trap with 6-inch [15 cm] legs made of wooden dowls) can be substituted for a dome if one is not available. The bait is placed in the bottom of a 6-oz. (0.2-L) styro-foam cup that has had all but the bottom inch (2.5 cm) of the cup trimmed away. The trimmed-down sytro-foam cup is suspended above the plastic cups with a short length of wire (see Figure 3).

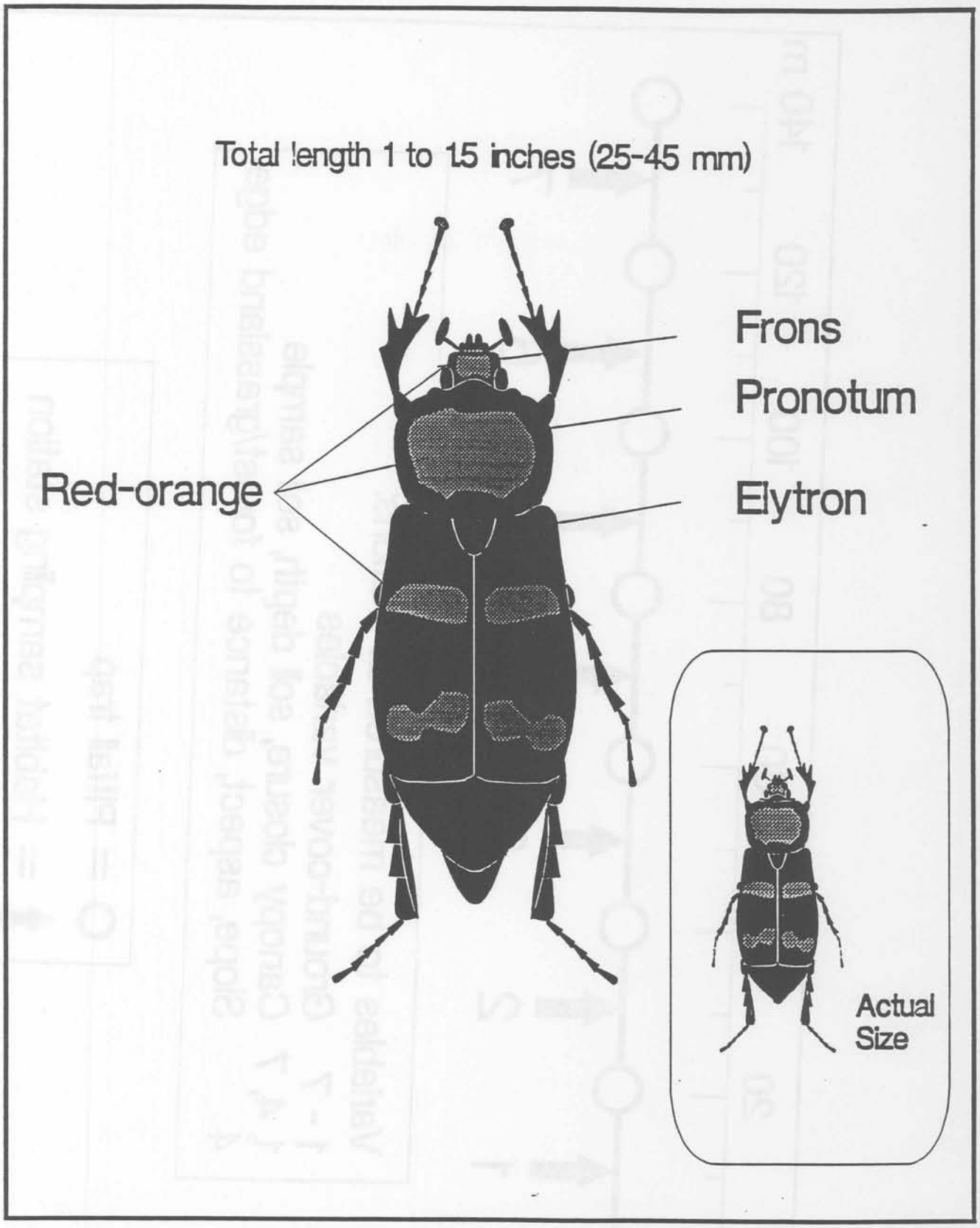

Figure 1. The Amerian burying beetle.

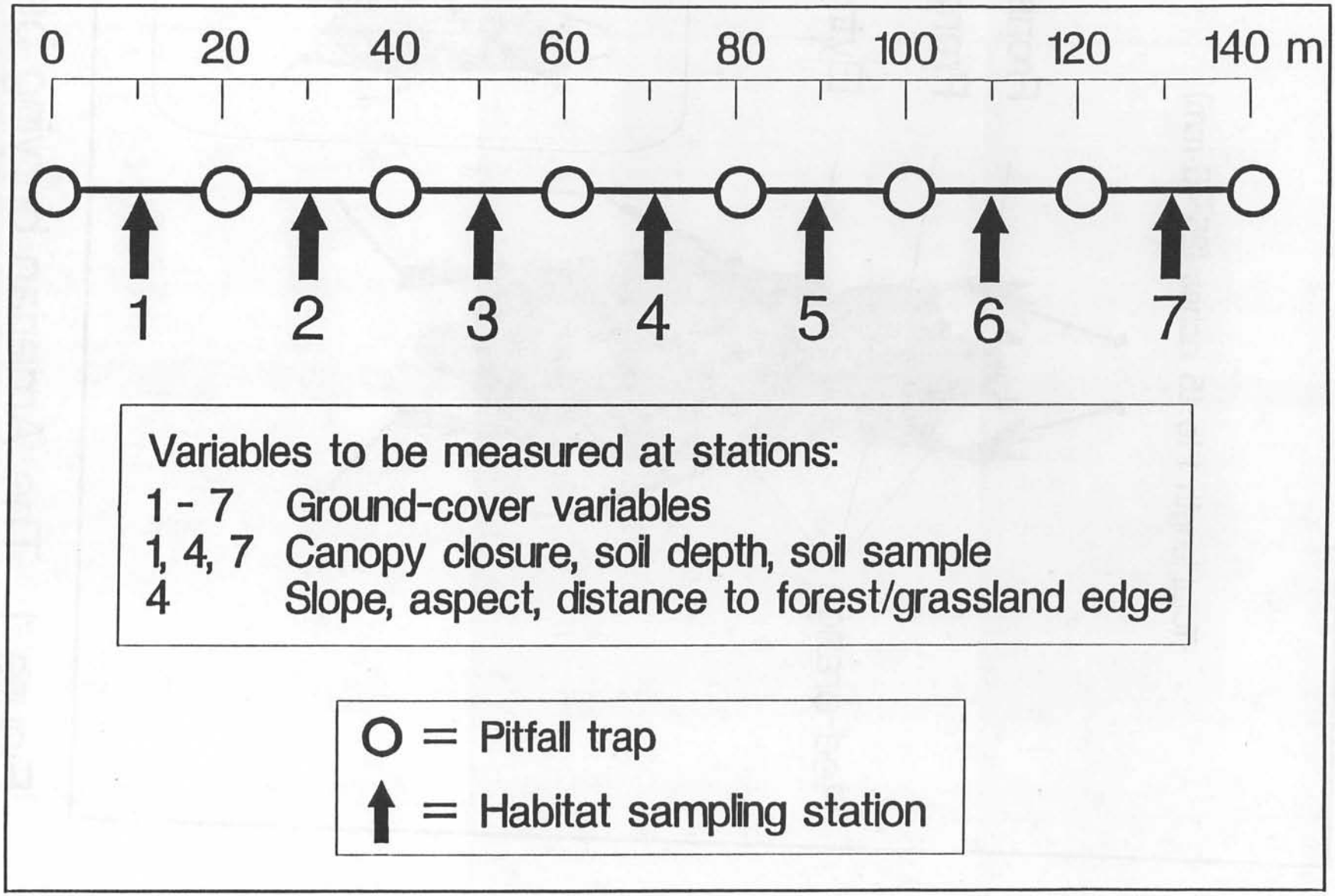

Figure 2. Diagram of survey transect.

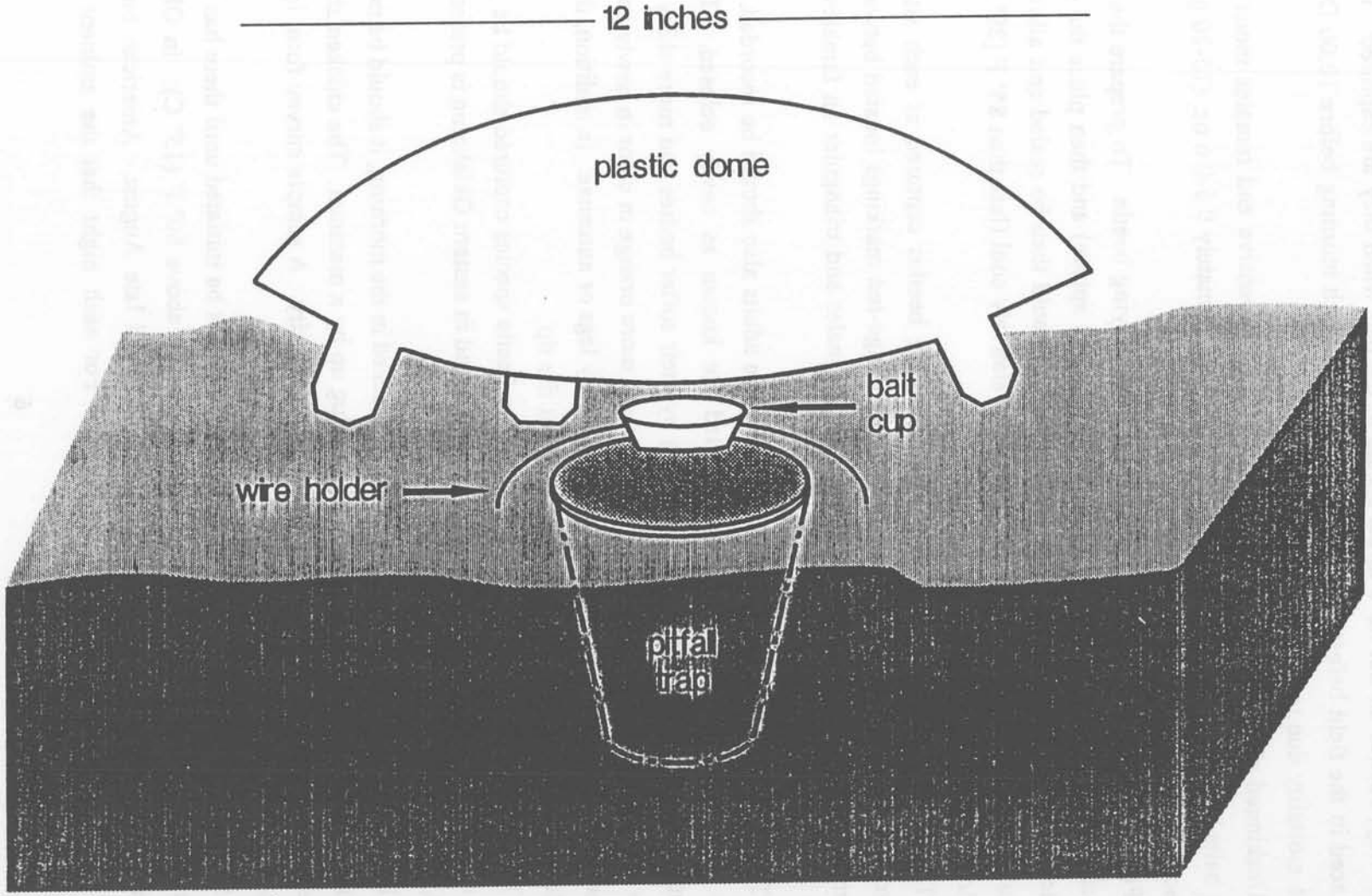

Figure 3. Diagram of pitfall trap.

In this way, beetles do not have direct access to the bait when they are captured. Traps should be placed in the field before 17:00 DST and checked each morning before 10:00 DST to avoid beetle mortality due to excessive heat.

Unskinned chicken is the preferred bait. It is inexpensive and remains moist longer than other baits because most of its fat is in the skin. Approximately 0.5-0.6 oz. (15-20 g) of chicken is placed in each pitfall trap.

Fresh bait is not an effective attractant of any burying beetle. To prepare the chicken for use, chop it up into small cubes (0.5-0.6 oz. [15-20 g] apiece) and then place the cubes into a plastic jar. Do not fill the jar completely. The jar should then be sealed and allowed to sit in the sun for a minimum of one day. If the day is relatively cool (less than 85° F [29° C]), the bait should sit in the sun for a longer period of time.

The numbers of each sex of American burying beetles captured at each site should be recorded. The sexes can be separated based on orange-red markings located between the frons and mandibles: these markings are rectangular on males and triangular on females (see Figure 4).

The number of newly eclosed and reproductive adults also should be recorded. Adults that have recently (less than two weeks) pupated are known as newly eclosed. They can be distinguished from the previous year's young by their softer bodies and more shiny appearance. The red-orange pronotum appears to be lighter and more orange in color in newly eclosed adults. Older adults are often missing body parts, especially legs or antennae. In addition, the mandibles of older adults appear to be a bit more worn at the tip.

The numbers of individuals of other burying beetle species captured should be recorded. A written description of the burying beetle species found in eastern Oklahoma is presented in Table 1. An identification key is found in Table 2.

If a pitfall trap is disturbed prior to being checked in the morning, it should be noted whether the trap was: intact but with bait missing; or dug up by a mammal. The chicken should always be replaced if it is taken during the night or becomes dry. A sample survey form is included in the appendix.

Surveys for the American burying beetle should not be initiated until there has been a week where minimum temperatures have been consistently above 60° F (15° C). In Oklahoma and Arkansas, we conduct surveys between mid-May and late August. American burying beetle activity is influenced by weather conditions. For each night that the ambient temperature

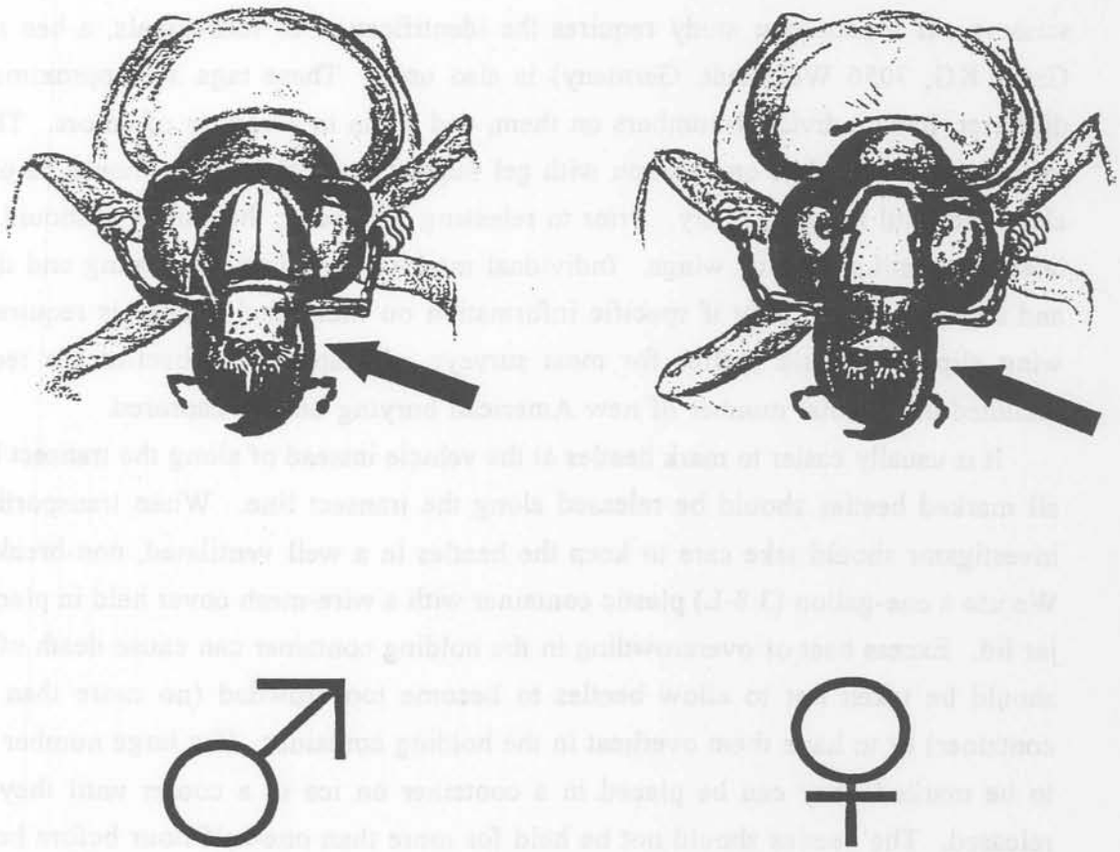

Figure 4. Characteristics distinguishing male from female American burying beetles.

dropsbelow 60° F (15° C) during the sampling period, the site should be sampled for another night. The site also should be retrapped if rainfall is heavy after dusk.

Marking Beetles

All American burying beetles captured need to be given a permanent mark by taking an eighth inch (3 mm), V-shaped clip out of the distal end of an elytron using small dissecting scissors. If a particular study requires the identification of individuals, a bee tag (from Chr. Graze KG, 7056 Weinstadt, Germany) is also used. These tags are approximately 1-mm in diameter, have individual numbers on them, and come in a variety of colors. The tag is glued to the proximal end of one elytron with gel Super Glue. The beetle should be placed in a dry, clean tub until the glue is dry. Prior to releasing the beetle, the surveyor should make sure the beetle can still spread its wings. Individual marking is a time-consuming and delicate process and should be done only if specific information on individual beetles is required. Otherwise, wing clipping should suffice for most surveys. Recaptures of beetles are recorded but not included in the total number of new American burying beetles captured.

It is usually easier to mark beetles at the vehicle instead of along the transect line. However, all marked beetles should be released along the transect line. When transporting beetles, the investigator should take care to keep the beetles in a well ventilated, non-breakable container. We use a one-gallon (3.8-L) plastic container with a wire-mesh cover held in place with a mason jar lid. Excess heat or overcrowding in the holding container can cause death of a beetle. Care should be taken not to allow beetles to become too crowded (no more than 10 beetles per container) or to have them overheat in the holding container. If a large number of beetles need to be marked, they can be placed in a container on ice in a cooler until they are marked or released. The beetles should not be held for more than one-half hour before being released.

Accidental Death of Beetles

The handling of all endangered species is strictly regulated by the United States Fish and Wildlife Service. When surveying for American burying beetles, a copy of the federal permit should be in your possession at all times. The handling of dead American burying beetles also is under strict regulation. They cannot be added to a private collection and only the United

States Fish and Wildlife Service is authorized to determine the proper disposition of beetles killed during surveys.

All American burying beetles killed during surveys need to be accounted for and an accidental-death form needs to be filled out as quickly as possible (see sample form in the appendix). The following information is to be noted:

(1) date beetle found dead;

(2) county, state, legal description (township and range) and any other information concerning location (i.e. trap number, site number or survey name);

(3) general habitat;

(4) as accurately as possible, the cause of death (previously, causes of death have included heat exposure, predation, and drowning);

(5) sex and age of beetle (whether it is a newly eclosed or reproductive age adult);

(6) name of individual that found beetle.

At a later time, the master permittee will note where the beetle was deposited. If the specimen cannot be prepared immediately, it should be placed in a sealable, rigid plastic container so the beetle is not crushed. To avoid mixing up specimens, no more than one beetle should be kept in a container. Each accidental-death form has a specimen number. A copy of this number should be placed in the container with the beetle so specimens do not become mixed up. The container should then be put on ice until the beetle can be prepared. Specimens are to be placed in the care of the field supervisor and then reported to the United States Fish and Wildlife Service as soon as possible.

Recording Habitat Variables

Habitat data are collected at all sites surveyed for the American burying beetle, including sites where American burying beetles were not captured. Each transect has seven habitat sampling stations with one station half-way between each pair of adjacent pitfall traps (traps are 20 m apart). Figure 2 illustrates where the sampling stations are located along the survey transect and lists data to be collected at each station. A sample habitat-data form is found in the appendix.

A habitat-sampling station is considered to be the area within a 0.5 x 0.5 m wooden frame placed on the ground. The investigator notes the presence of grasses, herbs, mosses, rocks, leaf litter, shrubs less than or equal to 2 m in height, shrubs greater than 2 m in height and woody

vegetation with dbh (diameter breast height) greater than 10 cm at each of the seven sampling stations.

Percent of canopy closure (to nearest percent), soil depth (in decameters), and a soil sample should be taken at stations 1, 4, and 7. Canopy closure is measured with a spherical densiometer (concave Model C, Forestry Suppliers, Inc., Jackson, Mississippi). The procedure follows Lemmon (1957) and is outlined on the inside cover of the densiometer (copy is included as Appendix 2). A soil sampler (Oakfield Apparatus Co., 19" tube sampler, Forestry Supplier, Inc., Jackson, Mississippi) is used to measure the depth of the soil, as well as to collect the soil sample. The three soil samples from each site are placed in a single spunbound olefin sampling bag (4.5 x 6 inch [11 x 15 cm, Ben Meadows Co., Atlanta, Georgia]) with the number of the survey site recorded on the bag.

The remaining data should be collected at station 4 only. The slope of the terrain is measured with an Suunto optical reading clinometer (Model PM-5-360 PC, Forestry Suppliers, Inc., Jackson Mississippi), and the slope's aspect is measured with a compass (in degrees from magnetic north). The distance to the nearest forest edge (recorded in grassland sites) or to edge of an open area (recorded in forested sites) is measured in meters with a rangefinder (Ranging Measuring Systems, Model 620, Forestry Suppliers, Inc., Jackson, Mississippi). At a site with scattered trees and open areas (such as a savannah), the distance is recorded as zero.

Reporting of Survey Results

The results of surveys for American burying beetles are sent to the United States Fish and Wildlife Service in Tulsa, Oklahoma and the Oklahoma Natural Heritage Inventory (Oklahoma Biological Survey) in Norman, Oklahoma.

References

Lemmon, P. E. 1957. A new instrument for measuring forest overstory density. J. Forestry 55:667-668.

U.S. Fish and Wildlife Service. 1991. American burying beetle recovery plan. U.S. Fish and Wildlife Service, Newton Corner, Massachusetts.

TABLE 1. Description of burying beetles found in eastern Oklahoma and western Arkansas.

Nicrophorus americanus: Four elytral spots. Orange-red pronotum and frons distingiush this species from all other North American burying beetles.

Nicrophorus orbicollis: Black pronotum with some texturing to it. Four orange spots on elytra (two/elytron) that do not extend to edges of elytra. Typically, it is the most common species in wooded habitats.

Nicrophorus marginatus: Similar to *N. orbicollis* except that each pair of elytral spots on is connected along lateral edge of elytron. Species found almost exclusively in grassland areas.

Nicrophorus sayi: Very similar to *N. orbicollis* except femur of each back leg is distinctly curved instead of straight. Also, proximal pair of elytral spots extend to lateral edge of the elytra. Active in early spring and quite rare after late June.

Nicrophorus tomentosus: Pronotum covered with fine, golden hairs. Easily distinguished from all other species by this characteristic. Found in variety of habitats.

Nicrophorus pustulatus: Relatively dark appearance with faint or absent elytral spots. Four small, orange spots may be visible at distal end of elytra (two spots/elytron).

Nicrophorus carolinus: Similar to *N. orbicollis* except that pronotum very smooth and domelike. Usually found near large rivers.

Table 2. Key to burying beetles of eastern Oklahoma and western Arkansas.

1A. Pronotum covered with fine, golden hairs..... *tomentosus*
1B. Pronotum not covered with hairs..... 2

2A. Pronotum and frons red-orange..... *americanus*
2B. Pronotum and frons black..... 3

3A. Elytral spots faint or absent..... *pustulatus*
3B. Elytral spots present..... 4

4A. Pronotum round, smooth and domelike..... *carolinus*
4B. Pronotum not round, smooth or domelike..... 5

5A. Femur of back leg distinctly curved..... *sayi*
5B. Femur of back leg straight..... 6

6A. Spots on each elytron connected on lateral edge of elytron..... *marginatus*
6B. Elytral spots distinct..... *orbicollis*

Appendix 1

1992 AMERICAN BURYING BEETLE SURVEY FORM; FORT CHAFFEE, ARKANSAS

Plot Number:_____ **Survey Night: 1 2 3** **Time (DST):**_____ **Date:**_____
D M Y

Surveyor:_____ **Habitat Description:**_____

Temp: Min_____/**Max**_____(°F) **Wind**_____**mph** **Cloud Cover (%)**_____

Trap No.	*americanus*	*orbicollis*	*tomentosus*	*pustulatus*	*marginatus*	Other
1 P GTI GDU						
2 P GTI GDU						
3 P GTI GDU						
4 P GTI GDU						
5 P GTI GDU						
6 P GTI GDU						
7 P GTI GDU						
8 P GTI GDU						
TOTALS						

MALE OLD _____
NEW _____
UNK _____
FMALE OLD _____
NEW _____
UNK _____

Newly Marked Males_____

Newly Marked Females_____

Recaptures:_____

COMMENTS:_____

Max/Min temp for previous 24-hour period; other weather data refer to current conditions. Date and time refer to when trap checked; P = Bait present; GTI = Bait gone trap intact; GDU = Bait gone trap dug up; OLD = breeding adult; NEW = newly eclosed adult; UNK = age cannot be determined. Newly marked males and females refers to color, number of bee tag, and age of beetle (e.g. R54[old]). Recaptures refer to color and number of bee tag on beetles that have been previously marked.

AMERICAN BURYING BEETLE ACCIDENTAL-DEATH FORM

SPECIMEN NUMBER_____

DATE FOUND:_____

Day Month Year

SITE DESCRIPTION

COUNTY:_____

STATE:_____

LEGAL DESCRIPTION OF SITE:_____

OTHER INFORMATION ON SITE LOCATION:_____

HABITAT DESCRIPTION:_____

_____

BEETLE DESCRIPTION

CAUSE OF DEATH:_____

_____

SEX: MALE FEMALE

AGE: NEWLY ECLOSED OLD UNKNOWN

OTHER COMMENTS:_____

_____

OTHER INFORMATION

COLLECTOR:_____

WHERE DEPOSITED (to be completed by master permittee):_____

_____

1993 AMERICAN BURYING BEETLE HABITAT-DATA FORM

SITE INFORMATION(Make sure same as on burying beetle survey form)_____

_____OBSERVER:_____DATE:_____

HABITAT:_____

Sampling Station 1.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

CANOPY N____ E____ S____ W____ **SOIL DEPTH(DM):** 0 >0-1 >1-2 >2-3 >3-4 >4

Sampling Station 2.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

Sampling Station 3.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

Sampling Station 4.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

CANOPY N____ E____ S____ W____ **SOIL DEPTH(DM):** 0 >0-1 >1-2 >2-3 >3-4 >4

DISTANCE TO EDGE FOREST/OPEN(M): _____**ASPECT:** _____**SLOPE(%):**_____

Sampling Station 5.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

Sampling Station 6.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

Sampling Station 7.

GRASS	HERB	LITTER	ROCK	SHRUB	TREE	MOSS
PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT
ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT
				≤2 m >2 m		

CANOPY N____ E____ S____ W____ **SOIL DEPTH(DM):** 0 >0-1 >1-2 >2-3 >3-4 >4

Appendix 2

Reprinted from the JOURNAL OF FORESTRY, Vol. 55, No. 9, September 1957

A New Instrument for Measuring Forest Overstory Density[1]

A new instrument called a "spherical densiometer" has been described for estimating forest overstory density.[2] This pocket-type instrument employs a mirror with spherical curvature which makes possible the reflection of a large overhead area. A grid is used to estimate percentage of this overhead area covered with forest canopy. Estimation is usually from a point near the forest floor. Adequate sampling gives the average canopy of a forest area.

Two models, A and B (Figs. 1 and 2), have been adopted as standard. Each employs a highly polished chrome mirror 2½ inches in diameter and having the curvature of a 6-inch sphere. The convex side of the mirror is used in Model A and the concave side in Model B. Each has some advantages over the other.

The mirrors are mounted in small wooden recessed boxes with hinged lids similar to compass boxes. The over-all dimensions are about 3½ x 3½ x 1⅛ inches. A circular spirit level is mounted (recessed) beside the mirrors. Positive slide fasteners are provided in Model B which allow the lid to open to an angle of about 45 degrees.

Cross-shaped and circular grids with squares and dots are used to estimate overstory coverage by tree crowns. Grids are of two kinds: (1) those scratched upon the surface of the mirror, Model A, and (2) those superimposed between the mirror and the eye, Model B.

The cross-shaped grid scratched upon the convex surface of the mirror in Model A has 24 quarter-inch squares (Fig. 3A). Instructions for using the densiometer and cumulative values for the squares on the grid are shown on a chart that is attached to the inside of the box lid (Fig. 3B). It is easier and faster to estimate the relative amount of overstory coverage with this instrument by assuming the presence of four equi-spaced dots in each square and by counting dots representing openings in the canopy. The percentage of overstory density is then assumed to be the complement of this number. Each assumed dot is assigned a value of one percent in this case. A slight discrepancy exists between estimations using the squares and estimations by counting assumed dots, because there are only 96 dots in the entire grid area. Cumulative values of the squares shown in the chart add up to 100 percent for the entire area within the grid. If desired, one may calculate the exact percentage values for each assume dot and thereby make the tv methods of use exactly comparabl

Model B has a circular grid. T circle is 1½ inches in diamet superimposed over quarter-in squares. Each square has four equ spaced dots (Fig. 4A). This gr is made from a positive print a photographic film mounted b tween thin sheets of plexiglass a fitted into the window of the b lid. Instructions for operati Model B are given on a cha mounted on the bottom of the strument box (Fig. 4B). The op ator estimates overstory dens by counting the dots representi overstory openings and assumi this to represent the percentage noncovered overstory area. He again a slight discrepancy exi because there are only 96 dots cluded within the area of circular grid. Exact percent values for each dot may be c culated to estimate the ent circular area as 100 percent. T refinement is not considered nec sary for ordinary use of the inst ment.

Instruments can be develop with different kinds, sizes. a shapes of grids and with mirr of different curvatures. Howev standardization of these proper is necessary to provide compara information that can be duplica The instruments described h been thoroughly tested and h given satisfactory results with m western conifers. We believe spherical densiometer descri (either Model A or B) will se

[1]*Editor's note.*—At the request of the author the reader's attention is called to the comercial availability of this instrument. See page 696.

[2]Lemon, Paul E. 1956. A spherical densiometer for estimating forest overstory density. Forest Sci. 2:314-320.

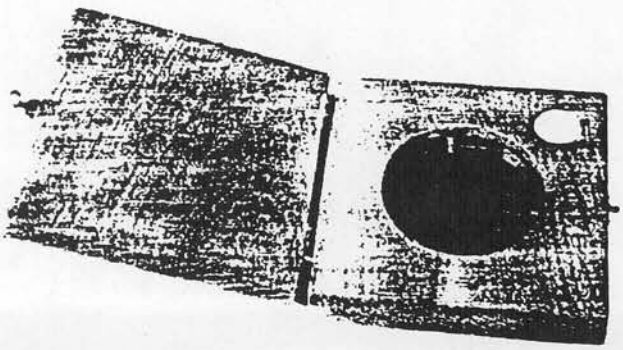

FIG. 1.—Spherical densiometer, Model A, with estimating grid scratched on the surface of the convex mirror.

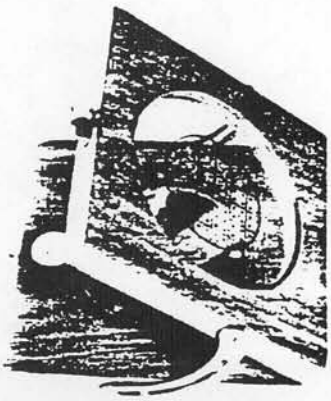

FIG. 2.—Spherical densiometer, Model B, with estimating superimposed between the eye and the surface of the con mirror.

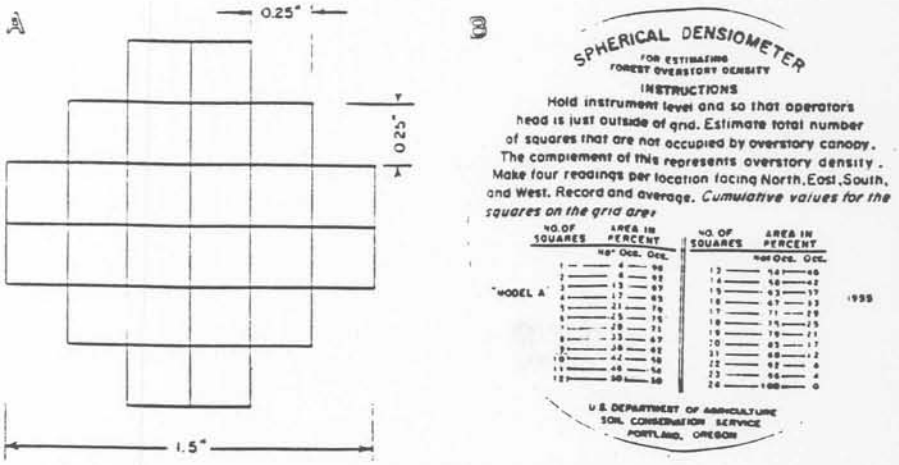

FIG. 3.—(A) Cross-shaped grid scratched on the convex surface of the mirror in Model A. Each square is ¼ inch on a side. (B) Instructions for using Model A. This is fastened to the inside of the lid of the mounting box.

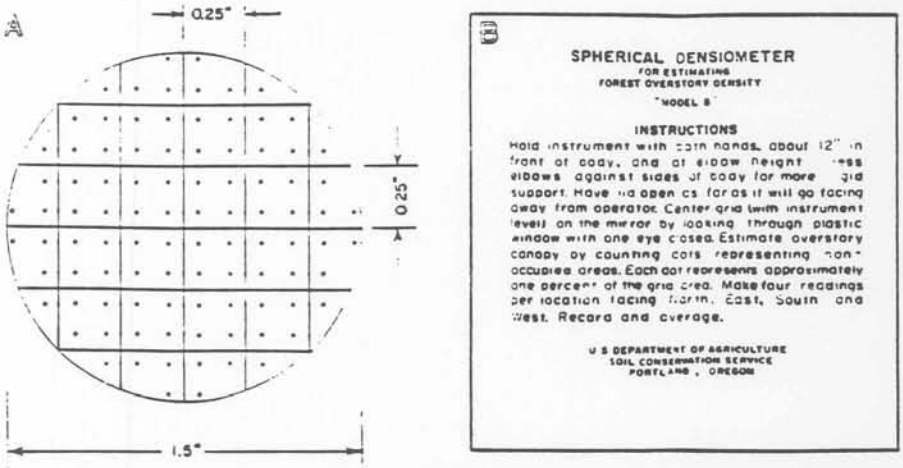

FIG. 4.—(A) Circular grid superimposed between the eye and the concave mirror in Model B. Each square is ¼ inch on a side. (B) Instructions for using Model B. This is fastened to the bottom of the mounting box.

the needs of practicing forester, range conservationist, and plant ecologist or those of most scientists doing highly technical work.

Operators need a little training to become consistent in the use of the instrument. Judgment and experience is needed to differentiate between overstory areas that are considered completely covered by the overstory and those that have thin but uniformly distributed coverage. In the latter case it may be necessary to estimate the area of many small irregular openings and reduce the percentage overstory density by the sum of these. Training and experience are needed for each different forest species or type because of the differences in overstory characteristics. The season of the year is important when making measurements in forests containing deciduous species.

Experience has shown that sufficient accuracy can be attained with the spherical densiometer by holding it as nearly level as possible in the hand. This is made possible by installing a circular spirit level in the mounting box. No mechanical support, such as a tripod, is needed. This adds to the practicability of the instrument in use.

A large number of measurements of overstory density have been made to test the instrument. One such study involved the measurement of overstory density at points in 28 different forests. Measurements were made at each point by four different operators each using instrument Model A and Model B. The results were subjected to an analysis of variance to determine consistency of measurements. There were no significant differences among measurements made by different operators or with different instruments and none of the interactions were significant. The differences due to forests, however, were highly significant—above the 99 percent level of probability. Under similar conditions one can expect variations in overstory density measurements to be within ±1.3 percent, ±2.4 percent and ±3.1 percent at probability levels of 70, 95, and 99 percent respectively. These variations amount to about 2, 3, and 4 percent when the standard deviation is expressed in terms of the overstory at the point of measurement (coefficient of variation).

Another study involved placement of 416 different forest overstory measurements into 5 percent overstory density classes. Variation around the mean within each class was calculated and the standard deviations and coefficients of variation plotted against the overstory density classes. It was found that variation among measurements increased as the overstory being measured decreased — only slightly when overstory density decreased from 100 down to about 60 percent but rapidly thereafter. When placing overstory density into 5 percent classes with the spherical densiometer, reliability in the order of about 5 percent can be expected so long as one is measuring forests that have more than about 50 percent overhead canopy. Since one naturally estimates percentage of overstory area not covered in dense forests and overstory area *covered* in open forests, estimations of overstory density when placed in classes will seldom vary more than ±5 percent.

Loss in reliability of overstory density measurements results from placing forests in overstory density classes based on measurements with the spherical densiometer as contrasted with using the actual measurements. For instance, reliability of about ±1.3 percent can be attained when actual measurements are used whereas the reliability is reduced to about 5 percent when classes are used.

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APPENDIX III

GENETIC VARIATION IN THE AMERICAN BURYING BEETLE, *Nicrophorus americanus*

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ABSTRACT

The American burying beetle, *Nicrophorus americanus* is a federally endangered species highly restricted in its distribution, although formerly widespread throughout the central and eastern United States. Reintroductions and translocations of American burying beetle populations will constitute an important component of the recovery program for this species. The goal of the present study was to provide information on the amount and pattern of genetic variation within and between two extant populations, Block Island and Oklahoma/Arkansas (OK/AR). Such genetic information on burying beetle populations is critically important to the design and implementation of a recovery program.

A genetic survey of *N. americanus* and a congener, *N. orbicollis* was performed using the Polymerase Chain Reaction with single short primers that randomly amplify polymorphic DNA (RAPD-PCR). The study on *N. orbicollis* compared a Block Island population with a nearby mainland population to determine whether the former group exhibited reduced variation due to its isolated location. The results demonstrated no obvious differences between the island and mainland groups indicating that the location of the Block Island population did not in itself reduce genetic variation.

The objective of our study on the endangered species was to characterize the genetic variation present in each of the two populations. Two questions were of primary importance; how much variation is present in Block Island and OK/AR, and does the variation differ between these two groups? The survey of *N. americanus* revealed low levels of genetic variation in both the eastern (BI) and western (OK/AR) populations. The OK/AR beetles did exhibit slightly more variation, both in the number of bands produced and in the combination of banding patterns present. There were no unique bands or banding patterns in the Block Island beetles. We therefore recommend the use of the OK/AR population for reintroduction and/or translocation programs implemented to accomplish the objectives outlined in the recovery plan for the reclassification of the American burying beetle.

INTRODUCTION

The maintenance of genetic variation in small populations of declining species is an important component of conservation biology (Frankel & Soule 1981, Oldfield 1984). A number of studies have been conducted using protein electrophoresis to examine allozyme variation as a means of characterizing genetic variation in extant populations of rare species (Triggs *et al.* 1989, Echelle *et al.*, 1989). Other studies have utilized methodologies that look directly at DNA variation including mitochondrial DNA and DNA fingerprinting (Ashley *et al.* 1990, Wayne *et al.* 1991, Vogler *et al.* in press). We have conducted a study using the Polymerase Chain Reaction to estimate genetic variability in two populations of the American burying beetle, *Nicrophorus americanus*.

N. americanus was widely distributed in the United States east of the Rocky Mountains as recently as 70 years ago. Records from pinned specimens indicate that this species occurred in 35 states and in three eastern Canadian provinces. The extant populations of this species are known only from locations on the periphery of their former range including Rhode Island, Oklahoma, Arkansas and Nebraska (just discovered in 1992). *N. americanus* was listed as an endangered species in 1989 (Federal Register 54:133, July 13, 1989) and a recovery plan was published in 1991 (U.S. Fish and Wildlife Service). The establishment of an extensive reintroduction and/or translocation program will most likely be necessary to meet the recovery objectives for reclassification as outlined in the plan. These objectives require the existence of three self-sustaining populations with a minimum of 500 individuals for at least five consecutive years in each of four broad geographic areas. Individuals from the known extant populations will serve as the source populations for captive breeding and reintroduction or translocation efforts. The goal of our study was to provide information on the amount and distribution of genetic variation among and between these extant populations to be used in the design and implementation of these programs.

An objective of our study on genetic variation in the Block Island population was to determine if the extant variation was a product of its island location or of declining population size. To distinguish island effects from small population effects, the best comparison would be to utilize samples from Block Island and from a close mainland population. This is not possible with *N. americanus* given their current distribution. However, we tested the "island effect" hypothesis by using the closely

related congener, *N. orbicollis* because it occurs on Block Island and in coastal Rhode Island. We assume that the migration rates of these two species were comparable when *N. americanus* was common in mainland areas close to Block Island. This report summarizes the results of our studies on both *N. americanus* and *N. orbicollis*.

MATERIALS AND METHODS

Collection of *N. americanus* from Block Island

Thirty-two beetles were removed as larvae from broods excavated on Block Island in 1990 and 1991. These individuals emerged as adults in the lab at Boston University, were bred in the captive colony and were frozen at -80°C. In addition seven beetles were removed from Block Island as adults in 1990, bred at Boston University and frozen at -80°C.

Collection of *N. americanus* from Oklahoma and Arkansas

In 1991 six adults from Latimer County, OK were received from Curtis Creighton, a graduate student at the University of Oklahoma. These individuals were used in the captive breeding program in Oklahoma, were frozen at -80°C, and shipped to Boston University on dry ice. In 1992 an additional 12 specimens were received. These beetles were mortalities that occurred in the course of pitfall trapping. Six specimens were from Fort Chaffee, AK, one was from Camp Gruber, OK (Cherokee/Muskogee Counties), and five specimens had no information at all. has indicated that he is unsure of the collection localities of the unmarked specimens. Seven of the 12 specimens received in 1992 were in reasonable condition and were used in our analysis. The five unlabelled specimens were in an advanced state of decomposition and were excluded from our study because they were contaminated with foreign DNA (i.e. microbes) and because they lacked reliable collection data. A total of 13 specimens (seven from OK and six from AK) were used in the analysis.

Collection of *N. orbicollis*

N. orbicollis were collected from pitfall traps on Block Island in 1990. All beetles from mainland Rhode Island were

collected in Matunuck in the same year. Specimens were transported alive to Boston University and frozen at -80°C.

DNA preparation

All DNA extractions were made on thoracic tissue dissected away from the exoskeleton and submersed in 500 ul of grinding buffer (10mM Tris-Cl pH 7.5, 60 mM NaCl, 10 Mm EDTA pH 8.0, 0.15 uM spermine, 0.15uM spermidine). This material was added to 500 ul of lysis buffer (0.2 M Tris-Cl pH 9.0, 30 Mm EDTA pH 8.0, 2% SDS) and digested with Pronase (0.2 mg/ml) at 37°C for one hour. Three extractions with phenol:chloroform:isoamyl alcohol (25:24:1, equilibrated to pH 8.0) and a final chloroform:isoamyl alcohol (24:1) followed. DNA was precipitated with 0.3 M sodium acetate in an equal volume of cold isopropanol. Samples were rinsed twice with 70% ethanol, vacuum dried, and resuspended in 75-200 ul of 1X TE. Aliquots of each sample were purified via spun column chromatography containing Sepharose CL-6B before being used in PCR reactions.

Polymerase Chain Reactions

The polymerase chain reaction is a technique by which small quantities of DNA can be amplified quickly and efficiently *in vitro* from total DNA preparations. PCR reactions consist of three major steps (Figure 1).

The first step, denaturation, is achieved by heating double-stranded DNA to a temperature of 94°C. At this temperature the bonds holding the two strands of DNA together dissociate and the DNA becomes single-stranded. This prepares the DNA for the second step, annealing. During the annealing phase, a small segment of DNA with a known sequence (the primer) attaches itself to the complementary sequence at the 5′ end of each strand of DNA (the template). Annealing temperatures range from 35-60°C depending on the specificity and length of the primers being used. The third step in the cycle, extension, reconstructs the complementary strands of the template DNA. The extension phase is accomplished with the use of a thermally stable enzyme called *Taq* polymerase. The template DNA acts as a reference strand for the *Taq* polymerase which adds complementary nucleotides along the entire stretch of DNA flanked by the primers. *Taq* polymerase operates most efficiently at a temperature of 72°C. This 3-step cycle is repeated 35-50 times. With each cycle doubling the amount of DNA from the previous cycle, a large quantity of DNA is generated quickly.

The primers used in our study are known as RAPD markers, which stands for Random Amplified Polymorphic DNA (Williams *et al.* 1990). This technique uses short primers, 10 bases in length, with arbitrary nucleotide sequences. The primers detect polymorphisms, or multiple alleles, which serve as molecular markers that can be compared across individuals to yield estimates of variation. The RAPD-PCR technique is similar to DNA fingerprinting in both the patterns of variation detected (Welsh and McClelland 1990) and in the analysis of those banding patterns (Gilbert *et al.* 1990). RAPD primers have recently been used in a number of studies to determine parentage (Scott and Williams 1993), to document hybridization (Crawford *et al.* 1993, Arnold *et al.* 1991), to identify strains of inbred mice (Welsh *et al.* 1991), to examine outbreeding in plants (Fritsch and Rieseberg 1992) and to determine the boundaries of a giant subterranean fungus (Smith *et al.* 1992). The use of RAPD primers to measure relative levels of variation within and between populations has not yet been reported in the literature.

PCR reactions were performed in a total volume of 25 ul containing 10 mM Tris-Cl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, and 120 mM each of dATP, dGTP, dCTP, dTTP. Approximately 25-30 ng of genomic DNA were used per reaction with 0.6 uM of a 10 base primer and 0.5 units of Promega™ *Taq* DNA polymerase. Forty-five primers were initially screened for use in this study. Primers that did not amplify any products or did not consistently amplify the same products were eliminated. Thirteen of these 45 primers were selected to conduct the survey of *Nicrophorus* beetles. Their names and sequences (5′ to 3′) are as follows: 211 (GAA GCG CGA T), 220 (GTC GAT GTC G), 222 (AAG CCT CCC C), 237 (CGA CCA GAG C), 272 (AGC GGG CCA A), 273 (AAT GTC GCC A), 275 (CCG GGC AAG C), 283 (CGG CCA CCG T), 284 (CAG GCG CAC A), 289 (ATC AAG CTG C), 290 (CCG CGA GCA C), 292 (AAA CAG CCC G), and 295 (CGC GTT CCT G). These primers were supplied by Dr. John E. Carlson and Dr. John Hobbs at the University of British Columbia's Nucleic Acid-Protein Service (NAPS) Unit as part of the Oligonucleotide Set #3. Three of the primers (211, 220, 222) were also synthesized by Dr. Dean Tolan at Boston University. Amplifications were performed in two MJ Research programmable thermal cyclers. There were no differences in banding patterns produced in the two machines, nor in different locations in the temperature block within either machine. PCR cycles were executed as follows: 1 min at 94°C (denaturing), 1 min at 35°C (annealing), and 2 min at 72°C (extension). Forty-five cycles were completed for each PCR reaction. Reaction mixtures containing all ingredients except template DNA were included as controls in most reactions. PCR products were resolved electrophoretically on 1.5% agarose gels at 75 volts for two hours and viewed with ethidium bromide staining and Polaroid™ photography. All reactions were replicated to confirm identical banding patterns in all individuals. Primers that did not

reproduce identical bands for all individuals within four replications were excluded from the analysis.

Southern Blot Hybridizations

Southern blots were conducted as controls to demonstrate allelism of representative bands. It was assumed that bands of the same length on the agarose gels were identical. In order to test this assumption, bands from a given primer in a single individual were used as probes against the same bands in other individuals. The products of RAPD-PCR reactions were run out on 1.2% agarose gels at 35 volts for 16 hours. The gels were transferred onto Zetabind™ nylon membrane according to the manufacturer's protocol. The bands used as probes were excised from 1.0% low melting point agarose gels and re-suspended in 50-100 ul 1X TE. This DNA was oligolabelled with ^{32}P and was hybridized to the nylon membranes at 65°C for 18 hours. The hybridized blots were washed to remove background signals and were exposed to x-ray film for 5-36 hours. Three bands were used for *N. americanus* and two for *N. orbicollis* as follows: a 1 kb band from primer 211, a 1.5 kb band from primer 283 and a 400 bp band from primer 284 for *N. americanus* and 300 and 700 bp bands from primer 295 for *N. orbicollis*.

Analysis of Banding Patterns

The measure used to estimate levels of variation in each population was as follows: for each primer we calculated the percent of shared bands between each pair of individuals within each population where $F = 2N_{xy}/(N_x + N_y)$. N_x and N_y are the number of RAPD bands in individuals *x* and *y* respectively; N_{xy} is the number of bands shared by *x* and *y*. The index is expressed as a percentage ranging from 0% (no shared bands) to 100% (identical bands in individuals *x* and *y*). This calculation of shared bands has been used in both restriction fragment length polymorphism (RFLP) and DNA fingerprinting analyses (Wetton *et al.* 1987). The Block Island individuals were divided into three groups so that all comparisons were made only on individuals run on the same gels. The sizes of the three groups were: group 1 (BI1) = 13, group 2 (BI2) = 12, and group 3 (BI3) = 14. The OK/AR specimens were run on gels together with 12 Block Island individuals representing each of the 3 Block Island groups (5 from BI1, 4 from BI2 and 3 from BI3). This allowed us to conduct a direct comparison of the percent of bands shared within each group as well as the percent of bands shared between Block Island and the western population.

RESULTS

1. Island Effect in *Nicrophorus orbicollis*

In the interim report submitted to the USFWS on 1/13/92, we presented results on *N. orbicollis* demonstrating that there were no significant differences in the percent shared bands between the Block Island and the Matunuck, RI populations. That study was conducted on 56 individuals (28 from each population) with seven RAPD primers. The present analysis was expanded to include the 13 RAPD primers subsequently used on *N. americanus*. We tested these 13 primers on 28 *N. orbicollis* individuals (14 from Block Island and 14 from Matunuck). The results were nearly identical to the first study and are presented in Table 1.

Twelve of the 13 primers were included in this analysis. Primer 273 was excluded because the banding patterns could not be replicated reliably. A total of 75 bands were analyzed for the Block Island group and 76 bands for the mainland group. An average of 6.3 bands were generated with each primer in both populations. Eight primers (211, 220, 222, 237, 272, 289, 290, 292) showed no obvious differences between the mainland and island populations. Three of these primers (211, 237, 289) were invariant in both populations. Two primers, 283 and 295, revealed considerably more variation in the Matunuck, RI population. The reverse was true for two other primers, 275 and 284, which revealed more variation in the Block Island population.

Two bands (300 bp and 700 bp) were excised from a Block Island individual and used to probe the Block Island and Matunuck groups. These markers clearly demonstrated identity of the bands they were tested against (Figures 2 and 3). All bands scored as present on the gels were present on the autoradiographs, indicating that the bands were homologous. Likewise, no bands that were scored as absent from individuals on the gels appeared on the autoradiographs indicating the absence of these bands and concordance between scoring of ethidium bromide stained gels and actual allelism.

TABLE 1 Percent shared bands within Block Island (BI) and Matunuck (Mat) populations of *N. orbicollis*

Primer	BI mean ± sd	Mat mean ± sd
211	1.00 ± 0.00	1.00 ± 0.00
220	0.58 ± 0.24	0.59 ± 0.24
222	0.69 ± 0.16	0.65 ± 0.18
237	1.00 ± 0.00	1.00 ± 0.00
272	0.50 ± 0.21	0.48 ± 0.22
275	0.75 ± 0.11	0.80 ± 0.11
283	0.91 ± 0.07	0.68 ± 0.22
284	0.91 ± 0.12	0.94 ± 0.09
289	1.00 ± 0.00	1.00 ± 0.00
290	0.80 ± 0.13	0.82 ± 0.16
292	0.54 ± 0.20	0.55 ± 0.23
295	0.87 ± 0.11	0.75 ± 0.14

2. Genetic Variation in *Nicrophorus americanus*

Ten of the thirteen primers were used in our analysis of *N. americanus* from Block Island and OK/AR. Three primers (220, 222 and 290) were excluded from our analysis because they did not reliably reproduce identical banding patterns in all individuals. A total of 33 bands were analyzed for the Block Island population and 37 bands for the OK/AR group. An average of 3.3 bands per primer were generated in the Block Island group and 3.7 bands in the OK/AR group. Table 2 shows the results of the percent shared bands within the three Block Island groups of *N. americanus*. Three of ten primers (211, 273 and 292) were invariant in all groups. The other primers ranged from 83.9% to 98% of all bands shared in common.

The results of the Block Island and OK/AR comparison are shown in Table 3. The first column shows the percent of bands shared within the 12 Block Island individuals run on the same gels as the OK/AR beetles. The results of the variable primers within the OK/AR population are shown in column two and range from 81.2% to 97.8% shared bands. The third column presents the percent of bands shared between these two populations.

TABLE 2 Percent shared bands within Block Island *N. americanus*

Primer	BI1 mean ± sd	BI2 mean ± sd	BI3 mean ± sd
211	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
237	0.93 ± 0.07	1.00 ± 0.00	0.98 ± 0.05
272	0.90 ± 0.08	0.84 ± 0.13	0.88 ± 0.12
273	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
275	0.96 ± 0.06	0.96 ± 0.07	0.93 ± 0.07
283	0.98 ± 0.05	0.98 ± 0.05	0.98 ± 0.05
284	0.98 ± 0.05	1.00 ± 0.00	1.00 ± 0.00
289	1.00 ± 0.00	0.94 ± 0.07	1.00 ± 0.00
292	1.00 ± 0.00	1.00 ± 0.07	1.00 ± 0.00
295	0.91 ± 0.15	0.84 ± 0.17	0.91 ± 0.15

TABLE 3 Percent shared bands between the Block Island and Oklahoma/Arkansas populations of *N. americanus*

Primer	**Within BI** mean ± sd	**Within OK/AR** mean ± sd	**Between BI-OK/AR** mean ± sd
211	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
237	0.96 ± 0.07	0.82 ± 0.21	0.89 ± 0.16
272	0.83 ± 0.13	0.81 ± 0.21	0.83 ± 0.18
273	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
275	0.96 ± 0.07	0.89 ± 0.09	0.89 ± 0.09
283	0.96 ± 0.07	0.98 ± 0.05	0.97 ± 0.06
284	1.00 ± 0.00	0.96 ± 0.06	0.98 ± 0.05
289	0.98 ± 0.05	0.93 ± 0.13	0.95 ± 0.10
292	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
295	0.95 ± 0.12	0.82 ± 0.17	0.82 ± 0.17

In addition to examining the percent of bands shared in common within and between these two populations, we also conducted an analysis of the banding patterns present in the two groups. In six of ten primers there were no differences between these two groups in the number of bands present, nor in the number of banding patterns among individuals. In the remaining four primers, the OK/AR population exhibited both a slightly larger number of bands and a slightly more diverse array of banding patterns than the Block Island group (Table 4, Figure 4).

As with the controls performed on *N. orbicollis*, the southern blot hybridizations conducted with *N. americanus* on

three bands from two primers indicated that the bands examined were homologous.

TABLE 4 *N. americanus* - RAPD patterns in Block Island and OK/AR

Primer	Block Island #bands/ # patterns	OK/AR # bands/ # patterns
211	2/1	2/1
237	4/2	5/4
272	5/4	6/4
273	2/1	2/1
275	4/2	5/3
283	4/2	4/2
284	4/2	4/2
289	4/2	5/3
292	2/1	2/1
295	2/2	2/2
TOTAL	33/19	37/23

DISCUSSION

The analysis presented above for *N. orbicollis* confirms the results presented in the interim report. We found no difference in levels of variation present in the Block Island and mainland populations of this species. This absence of an island effect can be accounted for by several ecological factors. It is possible that migration from mainland source populations to Block Island occurs frequently enough to homogenize variation between them. Given that Block Island is only twelve miles from southern Rhode Island, and that very low, but consistent, migration rates are sufficient to homogenize populations, this scenario is plausible. It is also possible that the founding population on Block Island was large, or carried large amounts of genetic variation that has been maintained over time.

The use of PCR and RAPD primers in our analysis of *N. americanus* has revealed a limited amount of genetic variation in both of these populations, albeit slightly more in the western group. Our analysis has focused on two questions to assess the levels of variation in this species. The first question addresses how much variation remains within each of these two populations. The analysis of bands shared within and between these two groups shows that in six of ten primers there is a lower percent of bands shared within OK/AR than between Block

Island and OK/AR. In three of ten primers the percents were identical and in only one of ten primers was the percent of shared bands slightly higher within the mainland than between the island and mainland populations.

The second question we have addressed is whether the extant variation in these two populations differs. If these two populations were depauperate in variation, but exhibited very different patterns, it would be critical to utilize both populations extensively in captive breeding and reintroduction programs. The isolation of populations on real or virtual islands frequently results in a decrease in levels of variation and marked differences in the identity of the variation present. In their study on Channel Island foxes using DNA fingerprinting, Gilbert *et al.* (1990) used the percent of shared bands as a measure of relative genetic variability in isolated populations of foxes on islands of varying sizes. They reported that variability was higher between island populations than within a population on a given island. Their analysis also enabled them to demonstrate a correlation between island size and band sharing with the populations on the largest islands having the highest levels of variability. Perhaps most interesting, Gilbert *et al.* (1990) were able to distinguish individuals from different islands based on unique diagnostic bands associated with different islands.

In our examination of the number of bands present and the nature of the banding patterns present, we have again detected only slight differences between the OK/AR and Block Island populations. The OK/AR population does exhibit somewhat more variation than the Block Island population. The question remains however, how do we account for the enormous similarities in the variation seen in these two widely separated populations?

There are a number of suggestions that can address this inquiry. It is possible that the American burying beetle is a species that has never carried high levels of genetic variation and that what we are seeing in the PCR analysis does not reflect a recent change in levels of variation due to a radical decline in population size, but the status quo for a long period of time. On the other hand, it is possible that the low levels of variation do reflect prior bottleneck events that may have been severe, during which the extant levels of variation declined significantly in consort with population size. If the latter hypothesis were true, we might still expect to have seen differences in the identity of the variation fixed in each population, instead of the strong similarities we have detected. An alternative explanation lies in the specific technique we have utilized to examine levels of variation.

While the potential applications of PCR and RAPD primers have been recently reviewed (Hadrys 1992), there is little information available on the precise regions of the genome targeted by RAPD primers. In standard PCR reactions, two primers are added to each reaction to amplify a specific segment of DNA flanked by the regions matching the sequence of the primers. In RAPD-PCR reactions, only a single, short primer is added to each reaction. These primers amplify segments of DNA flanked by areas that have an inverted repeat. In other words, the primer corresponds to a sequence that it matches on one strand of DNA that runs from 5′ to 3′ and it matches the inverse sequence on the other strand in the 3′ to 5′ orientation. The high levels of similarities in the variation detected in the Block Island and OK/AR populations may result from the fact that these inverted repeats in *N. americanus* are located in highly conserved regions of the genome. However, most studies using RAPD primers report yields of large numbers of bands per individual and we consider it very unlikely that the results we have obtained are an artifact of the methodology we have used.

RECOMMENDATIONS

A reintroduction program using Block Island individuals from the captive population at Boston University has been ongoing since 1990. This effort has been conducted on a single small island in Massachusetts, Penikese, and is being considered at a second island site, Nantucket, for the summer of 1993. The use of Block Island individuals for these reintroduction efforts is recommended for two reasons. First, any inherited local adaptations to environmental conditions on Block Island would be very similar to conditions encountered on these other islands. In addition, the impact of this program on the wild population on Block Island has been minimal because all founding individuals for the reintroduction program have come from a captive breeding program.

The population of *N. americanus* on Block Island is known to be small, numbering approximately 500 individuals (Kozol 1988, annual monitoring efforts). Recent studies in Oklahoma and Arkansas have shown that this population is more widely distributed (nine counties in OK, four in AR) than previously thought, and is larger than earlier estimates revealed. In 1992 over 1000 adults were captured in pitfall traps (Lomolino, personal communication). The Oklahoma/Arkansas population should be used to establish a large scale reintroduction/translocation program for mainland sites.

The only long term captive breeding program currently in existence is housed at Boston University and is based on Block Island stock. This population will only be maintained through the end of 1993. Unless an institution in the area expresses a willingness to provide a long term commitment to housing a captive colony of *N. americanus*, it is probably not feasible to maintain a captive breeding program based on this stock. The Cincinnati Zoo also houses a captive population based on Block Island stock, but has had ongoing difficulties establishing a successful breeding regime. We recommend that the Cincinnati Zoo initiate a captive breeding program using Oklahoma beetles as the founding individuals. The excess adults from this population can be used in reintroduction efforts throughout the former range of this species. A captive breeding program is also in place at the University of Oklahoma and should produce additional animals to be used in reintroductions.

As survey efforts continue in the central United States and additional information on the distribution and size of the population in this area becomes available, the decision to implement a translocation program can be made. The advantages of a translocation program are several. Reintroductions require the maintenance of a captive colony that can be very time consuming for the individuals providing care. In addition, captive breeding of burying beetles, when successful, tends to generate a large number of excess individuals that must be maintained under the conditions of the permit. Translocations can be conducted in a manner which minimizes the impact on wild populations by following the methods used by Kozol (1990) on Block Island to remove individuals for captive breeding and genetic analyses. Wild adults are provided with carrion in the field. On the tenth day after burial the brood chamber is excavated, a small number of larvae are removed and allowed to complete development in captivity. The adults that subsequently emerge are used as the founding individuals in the translocation program.

SUMMARY

The use of RAPD-PCR has revealed low levels of genetic variation in both the Block Island and Oklahoma/Arkansas populations of the American burying beetle. Given that the OK/AR population exhibits slightly more variation than the population on Block Island, we recommend using the former group for mainland reintroduction and/or translocation programs.

FIGURE 1 The PCR Cycle

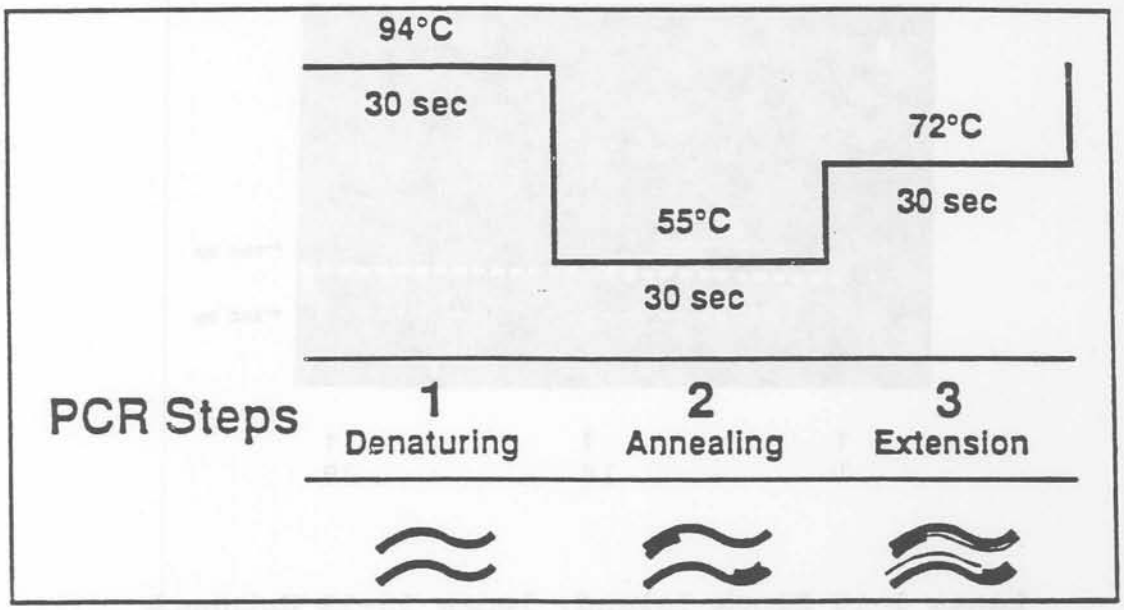
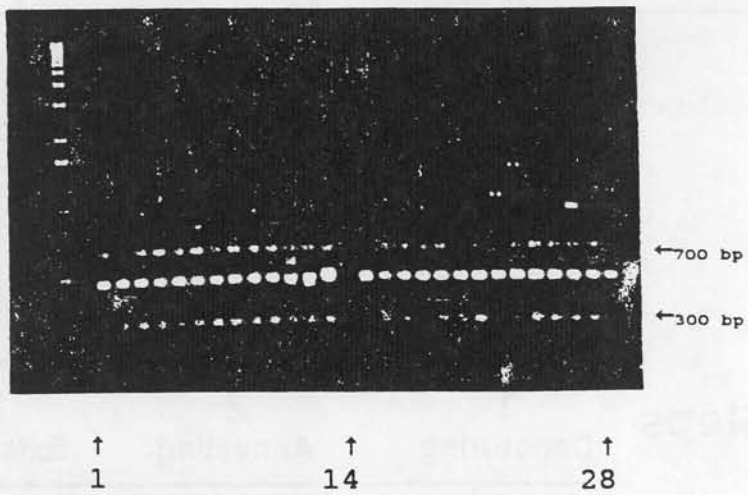
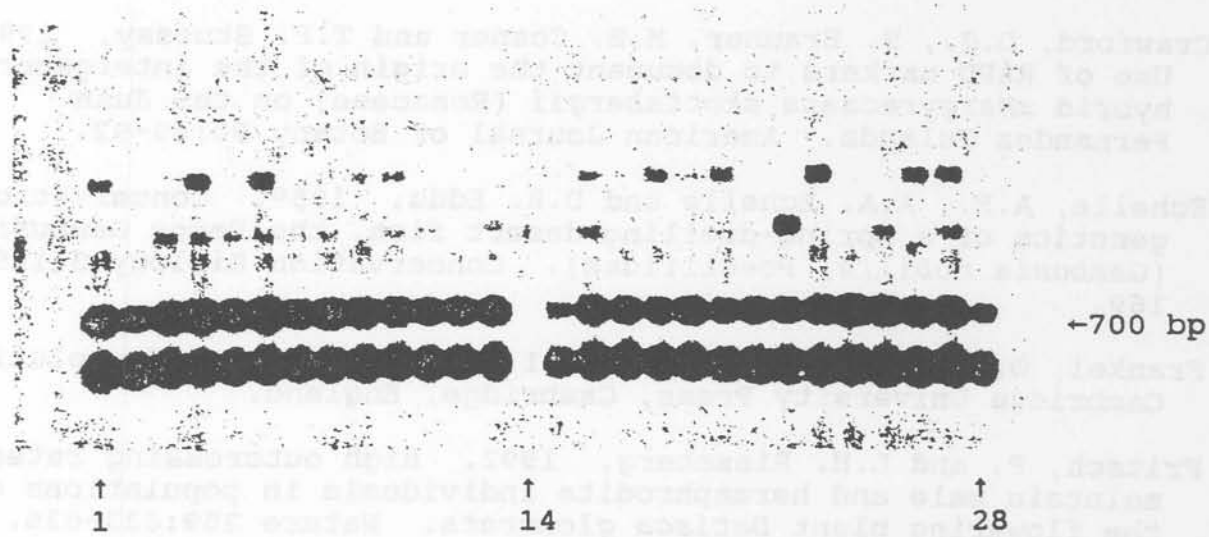


FIGURE 2 RAPD amplification products of *N. orbicollis* using primer 295.



Lanes 1-13 Block Island, lanes 15-28 Matunuck

FIGURE 3 **Autoradiograph of the gel from Figure 2 after probing with DNA from the 700 bp band, demonstrating that this band is allelic across all *N. orbicollis*.**



Lanes 1-13 Block Island, lanes 15-28 Matunuck

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