To the Honors Committee:

I am submitting this paper written by Michelle Ray entitled "Road Mortality of Mesocarnivores and the Relationship to Evolution: Natural Selection or Genetic Drift?" I have read the final form and content of this manuscript and recommend that it be accepted in partial fulfillment of the requirements for my Honors Project with a major in Biology.

Troy A. Ladine, Ph.D. Honors Committee Chair Professor of Biology

We have read this manuscript and recommend it acceptance:

J. Catherine Cone, Ph.D. Professor of Biology

Mark Miller, Ph.D. Associate Professor of Sociology Road Mortality of Meso-carnivores and the Relationship to Evolution: Natural Selection or Genetic Drift?

## Abstract

Mandibles of 33 raccoons, *Procyon lotor*, and six striped skunks, *Mephitis mephitis*, that died in collisions with motor vehicles were collected in Harrison County, Texas from 1 April 2012 to 1 October 2013. Age data were collected from the mandibles of raccoons using the age classes of Grau et al. (1970) and for skunks through cementum annuli technique (Feldhamer et al. 1982). Distribution of age classes for raccoons was 8:14:3:3:0. The distribution of age classes was analyzed for randomness using Chi-square analysis based on two expected distributions (a Gaussian distribution of the collected data and Ladine (1995). The distribution of road-killed raccoons in Harrison County, TX did not deviate from either expected distribution (Gaussian:  $\chi^2 = 0.042$ , p = 0.9998: Ladine (1995):  $\chi^2 = 0.123$ , p = 0.9972). Distribution of the collected data does not deviate from the expected indicating that road mortality of these species is most likely random with evolutionary consequences governed by genetic drift.

Conservation attempts to preserve genetic diversity (Schonewald-Cox 1983; Avise 1996). Roads can have a major impact on genetic diversity, primarily through changing gene flow (Fankel and Soulé 1981; Forman et al. 2003; Conard and Gipson 2006). The potential barriers created by a road can then potentially increase genetic drift with a population fragmented by the road (Wright 1931).

A second impact roads have on wildlife is through mortality caused by impacts with motor vehicles. This has the obvious effect of reducing population number. Most studies have

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investigated the influence roads have on fragmentation and gene flow (see Balkenhol and Waits 2009; Jackson and Fahrig 2011). Studies have shown population quality, primarily numbers, is influenced by vehicle influenced mortality (VIM; e.g. Caro et al. 2000; Bujocek et al. 2011). While Bujocek et al. (2011) investigated the possibility of selection on VIM in relation to birds, I am not aware of any investigation of VIM and its relationship to natural selection in mammals.

If selection is influenced by VIM, the diversity of a population could be directed toward animals avoiding roads. Thus, increasing the fragmentation effect (Wright 1931) roads have on the population and reducing gene flow within the population. However, if VIM is random a population may be less inclined to become fragmented by roads and retain gene flow within the population. The objective of this study is to investigate the importance of natural selection and genetic drift in relation to VIM. Selection should be occurring if selected age groups are subjected to higher rates of VIM than other groups.

Raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) make excellent organisms to test this objective. Both species are highly urban-adapted and have higher densities in both suburban and urban areas than other species (Gehrt 2003, Rosatte et al. 2010, Graser et al. 2012) making them more likely to come into contact with roads. These higher densities have been implicated in increased VIM (Conard and Gipson 2006). Additionally, they are large enough to be observed while driving reducing accidental exclusion from collection.

## MATERIALS AND METHODS

The study was conducted in Harrison, County Texas. The species collected for the study were raccoons and striped skunks. Most of the specimens come from the Highway 80 corridor between Hallsville, TX and Marshall, TX including the urban areas of both cities. This area

varies from a two-lane to a four-lane highway surrounded by forested area as well as pastures. Collection occurred from 1 April 2012 to 1 October 2013.

Data collected from animals were sex and mandible with teeth. Raccoons were aged using tooth-wear categories of Grau et al. (1970; Age I 0-14 months, Age II 15-38 months, Age III 39-57 months, Age IV 58-86 months, Age V  $\geq$  87 months).

The mandible for skunks, was boiled to release the premolars and the molars from the jaw. A Dremel rotary tool was used to sand the teeth to a thin section. Striped skunks were aged by the cementum annuli technique (Feldhamer et al. 1982). Due to the limited number of skunks (n=6), data for the species were not analyzed.

Data were tested for randomness by comparing the distribution of age classes to a Gaussian distribution established using Excel (Microsoft 2013) based on the collected data using a Goodness of Fit test. The Gaussian distribution provides a baseline for the testing. All data were analyzed using SPSS (IBM 2012). A second test for randomness was made using a known population in a mesic hardwood forest from Ladine (1995). While the population numbers in Ladine (1995) are not the actual population the sample was drawn, this test allows a comparison to a known population in a similar habitat and setting. Ladine (1995) collected data using markrecapture techniques in a population in a suburban setting 50 km north of Memphis, TN. Previous studies (Case 1978, Rolley and Lehman 1992) indicate that VIM is not a good indicator index of population densities. While Bujocek et al. (2011) assessed selection based on a comparison of VIM to predation of songbirds by raptors, this type of comparison is not possible for raccoons. Raccoons have few predators other than vehicles and hunting (Stuewer 1943, Whitney & Underwood 1952, Sanderson 1960, Johnson 1970) with the known predators in the study (i.e. bobcats, *Felis rufus*, coyotes, *Canis latrans*, great horned owls, *Bubo virginanus*; Whitney & Underwood 1952, Sanderson 1960, Johnson 1970) having low densities. These predators most likely did not kill many raccoons when they were more common (Kaufmann 1982). Mass to girth ratio has been shown not to be an indicator of fitness in the species (Ladine 1998). Although there is a correlation between kidney fat index and fitness in raccoons (Johnson 1970), either measure would be unreliable as an estimator of selection pressures of VIM because of the state of decomposition.

## RESULTS

Thirty-three raccoons and six striped skunks were collected during the study. Sex ratio of the collected raccoons is 0.83:1.0 (male: female) did not deviate from a 1:1 ratio ( $\chi^2 = 0.1366$ , P = 0.7116).

The mean age class of raccoons is  $2.2 \pm 0.9$ . The distribution of the age class of raccoons (Table 1) did not deviate from the Gaussian distribution ( $\chi^2 = 0.042$ , p = 0.9998) or the distribution of Ladine (1995;  $\chi^2 = 0.123$ , p = 0.9972). A Kruskal-Wallis test was run and determined there is no significant difference in ages of the sex groups (H = 0.037, P = 0.843). No age class V individuals were collected during the current study.

Table 1. Number of raccoons (*Procyon lotor*) involved in vehicleinduced mortality collected from 1 April 2012 to 1 October 2013 in Harrison County, TX. Age classes are those of Grau et al. (1970; I – 0-14 months, II – 15-38 months, III –39-57 months, IV – 58-86 months). There were no age class V individuals collected during the study.

Age Class	Number	Ladine	
Ι	8	80	
II	14	158	
III	8	116	
IV	3	64	

#### DISCUSSION

Sex ratio of collected raccoons was slightly biased toward females. This slightly contradicts some studies using mark-recapture techniques (Kaufmann 1982, Sanderson 1987, Ladine 1995, Gehrt & Fritzell 1996b). However, female biased sex ratios are not unknown (Gehrt & Fritzell 1996a, 1998). Females may be more likely to occupy habitat near roads than males. Intersexual habitat use disparity in raccoons is not known to occur and will require further study to confirm if this may result in a female biased sex ratio based on VIM.

Recognizing the limitations of estimating population age structure from a different population, the current study found no deviation between the population based on VIM and the distribution of a population in similar habitat (from Ladine 1995). The lack of deviation may be attributed to a random loss of individuals due to VIM in the current study. No age class was disproportionally represented in the sample, VIM most likely is not strongly controlled by factors involved with natural selection.

Additionally, animals were collected throughout an entire year with no spikes in age class collection except for Age Class I individuals. These raccoons are young-of-the-year individuals and will most likely be present in large number during the late summer to fall (July-October) when they are becoming independent of their mother (see Lotze and Anderson 1979, Gehrt 2003). The timing of the collection of Age Class I individuals in the current study could be a related to learning about roads without the presence of the mother. Thus, there may be a limited component to selection in the youngest age class.

Alternatively, VIM of raccoons observed in the present study may be attributed to speed of traffic and density of traffic. With one exception, all raccoons, regardless of age were found on roads with posted speeds between 88 and 105 km/h. The exception was an Age Class I raccoon located on a road with a posted speed of 56 km/h. Previous studies have found a correlation between speeds (Case 1978, Rolley & Lehman 1992, Caro et al. 2000) with an increase in VIM related to higher speeds. The road with the slowest speed in which the Age Class I raccoon was collected has a high density of traffic (pers. obser. Ladine). High density of traffic has the same correlation with VIM as speed (Case 1978, Caro et al. 2000).

Roads result in fragmentation of habitat for a considerable number of species. However, for meso-carnivores, fragmentation of habitat appears to be minimal (Oxley et al. 1974, Caro et al. 2000) with roads not serving as a barrier to separate populations. Nevertheless, populations of meso-carnivores can become fragmented by other means and VIM could become important in altering genetic diversity. The current study indicated selection probably plays a minor role in VIM. The majority of VIM appears to be random. Therefore, as long as populations are not

limited in effective size, VIM will not be a major influence in changing diversity within the population. However, in a population with a limited effective size, VIM could result in drift and loss of diversity. Further studies will need to be conducted to determine the amount of VIM induced drift will influence a population.

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# Appendix 1

- Table 1. Raccoon data collected from 1 April 2012 to 1 October 2013 in Harrison County, Texas. (Age I 0-14 months, Age II 15-38 months, Age III 39-57 months, Age IV 58-86 months, Age V  $\geq$  87 months).
- Table 2: Skunk data collected from 1 April 2012 to 1 October 2013 in Harrison County, Texas.(Age I 0-14 months, Age II 15-38 months, Age III 39-57 months, Age IV 58-86 months,<br/>Age  $V \ge 87$  months)

Specimen	Age Class	Sex	Date of Collection
1	III	Male	23 Apr 2012
2	IV	Male	7 Sep 2012
3	Ι	Female	5 Oct 2012
4	II	Female	4 Feb 2013
5	II	Male	24 Mar 2013
6	II	Male	26 Mar 2013
7	Ι	Female	10 May 2013
8	III	Male	29 May 2013
9	Ι	Female	29 May 2013
10	III	Female	29 May 2013
11	Ι	Female	29 May 2013
12	II	Male	20 Aug 2013
13	III	Female	13 Sep 2013
14	II	Male	13 Sep 2013
15	II	Female	13 Sep 2013
16	II	Female	13 Sep 2013
17	II	Female	14 Sep 2013
18	Ι	Male	14 Sep 2013
19	II	Female	18 Sep 2013
20	II	Female	20 Sep 2013
21	II	Male	20 Sep 2013
22	Ι	Male	20 Sep 2013
23	II	Female	21 Sep 2013
24	IV	Female	22 Sep 2013
25	III	Male	22 Sep 2013
26	III	Male	23 Sep 2013
27	Ι	Male	23 Sep 2013
28	II	Female	23 Sep 2013
29	III	Male	24 Sep 2013
30	II	Female	24 Sep 2013
31	IV	Female	28 Sep 2013
32	Ι	Male	1 Oct 2013
33	III	Female	1 Oct 2013

Table 2: Skunk data collected from 1 April 2012 to 1 October 2013 in Harrison County Texas. (Age I 0-14 months, Age II 15-38 months, Age III 39-57 months, Age IV 58-86 months, Age V  $\geq$  87 months).

Skunk	Age Class	Sex	Collection Date
1	Ι	Male	1 Dec 2012
2	IV	Female	28 Mar 2013
3	II	Male	30 Mar 2013
4	III	Female	30 Mar 2013
5	III	Male	8 Apr 2013
6	IV	Male	19 Aug 2013