Elevational and temporal variation in Ipomopsis floral and vegetative traits

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Abstract

Exploring the geographical and temporal movement of hybrid zones provides practical evidence for conservation biologists investigating the dynamics of species invasions (Buggs 2007). Genetically based clines in floral traits can also help us to understand potential adaptation in responses to climate change. We re-measured floral traits in *Ipomopsis* in 12 populations along an elevational gradient in Poverty Gulch, Gunnison County that was first measured in 1991 and 1992. Corolla length increased by 4.4 mm on average and corolla width by 0.11mm on average between 1992 and 2015. In a common garden experiment, we also found a genetic and environmental basis for variation in corolla length and anther position. Since there is a genetic component to the variation, the change in corolla length over 23 years could represent an evolutionary change rather than just a response to environment. Unlike corolla length, corolla width did not change systematically over time when supplementary data from additional years were compared. Additionally, anther position varied significantly only at the lower elevational sites between 1992 and 2015. Floral trait variation in this hybrid zone involves a complex assortment of selection mediated by multiple pollinators and varied environmental conditions that may be the cause of differential change in floral traits throughout the cline.

Introduction

The study of natural variation in floral traits is important to help understand the roles of natural selection, gene flow and genetic drift in shaping

levels of adaptation and distribution of species (Jonas & Geber 1999). Evidence of local adaptation demonstrates the significance of past evolutionary processes and suggests a key role in plant responses to current ecological dynamics (Pratt & Mooney 2013). Intraspecific clinal variation in plant traits has been observed along elevational gradients in environmental conditions (Vitasse et al. 2014). Consistent abiotic gradients have been shown to drive genetically based clinal variation in morphology and phenology within the same species (Pratt & Mooney 2013). However, quantitative variation can also be the result of plasticity and does not necessarily represent genetic variation.

Genetically based clines in floral traits can also help us to understand potential adaptation in responses to climate change. Even though local adaptation of plant species has been widely studied, there is less known about plasticity in plant traits and variation across species ranges (Leimu & Fischer 2008). A more variable environment should be associated with more variation in plasticity. One key spatial pattern is changes in specific traits of plants over elevation (Scheepens et al. 2010). A shaper change in trait with elevation could indicate stronger natural selection on that trait.

Our study system is two species of Ipomopsis (I. aggregata and I. tenuituba) and hybrids between them, which grow along an elevational gradient within Poverty Gulch, Gunnison County, CO. *Ipomopsis aggregata* grow in the valley at elevations of 2900 m and below, *I. tenuituba* can be found on steep

slopes above 3100 m, and natural hybrid populations grow on dry slopes at intermediate elevations between the two parental ranges (Campbell, 2004)..

Elevation differences within our study sites are associated with water availability, and vegetative and physiological traits, such as water-use efficiency, are often expected to experience different selection in dry versus wet habitats (Dudley 1996). However, instantaneous measure of water use efficiency (*A/E*), photosynthetic rate (A_{max}) or transpiration rate (*E)* showed no significant correlation with corolla length or width in a study at Poverty Gulch(Campbell et al. 2005). But in a later study at RMBL, *I. aggregata* generally increased flower production with increased moisture, and corolla length increased with greater water for all three plant types studied, *I. aggregata*, I. *tenuituba* and hybrids (Campbell & Wendlandt 2013). Also, average floral morphology did not change in a plastic way with snowmelt date (Campbell & Powers 2015). These data may indicate changes in elevational patterns over time could reflect both genetic differences and phenotypic plasticity. Genetic differences could be driven by natural selection or by temporal changes in the extent of hybridization. Differences in natural selection across the gradient could be produced by the differences in water availability or by differences in the abundance of pollinators, hummingbirds versus hawkmoths (Campbell et al. 1997).

Spatio-Temporal variation in selection has received relatively little attention, often due to a lack of long-term data (Siepielski et al. 2009). With a dataset dating back 20 years, the comparison of cline variation in floral traits could describe how these traits have evolved through time. Exploring the

geographical movement of hybrid zones also provides practical evidence for conservation biologists investigating the dynamics of species invasions (Buggs 2007). Evaluating causes and consequences of hybrid zone movement is critical if their long-term fate, the extinction of one taxon, is to be averted (Rhymer and Simberloff, 1996); many threatened species owe their demise in part to hybridization with invading relatives (Buggs 2007; Wolf et al. 2001; Allendorf and Lundquist 2003).

Plastic responses to precipitation have been shown to contribute to he process of natural selection, the process of speciation and macroevolution (Scheiner, 1993; Crispo, 2008). Specific leaf area (SLA=leaf area/dry mass) has been shown to plastically change in response to altered precipitation (Knight and Ackerly, 2003), an adaptive response to variation in water availability. Plants growing under dry conditions are likely to have a lower SLA, as greater surface area also results in increased water loss through transpiration. However, increased surface area also allows for maximized light collection and gas exchange, and therefore can be an advantage when water is not a limiting resource (Cornelissen et al., 2003).

This study began by addressing the following question. 1. How do present day elevational clines in corolla length, corolla width, and nectar production compare with those observed in 1991 and 1992 (Campbell et al. 1997) for populations of Ipomopsis located in Poverty Gulch, Gunnison County, Colorado? To supplement these data, SLA was collected to compare a cline in a vegetative trait with clines in floral traits in 2015. Based on known patterns of specific leaf

area, we predicted that populations of *Ipomopsis* will exhibit higher SLA in sites with wetter soils, and lower SLA in sites with drier soils. I hypothesized that selection on vegetative clines is expected to differ from that on floral clines, because environmental conditions are not extremely varied among populations along the elevational cline, but floral selection pressures are determined by three pollinators that are inconsistently present from year to year.

To explore the potential causes of temporal shifts in clines, we then used ongoing common garden experiments to answer the following: 2. To what extent are elevational differences in corolla length and width due to phenotypic plasticity versus genetic differences? Based on known patterns of floral traits, we predict that populations of *Ipomopsis* will exhibit relatively short and wide corollas in the species *I. aggregata* in lower elevations, while the species *I. tenuituba* expresses longer narrow corollas at higher elevation (Campbell 2004).

Methods

Study System

Observations were collected from 12 populations (A-L) along a 3.7km transect in Poverty Gulch, Gunnison County, CO (Fig. 3). Three of these sites exist as part of a common garden study, while the remaining nine are representative only of natural populations. This mountainous valley contains natural populations of *I. aggregata* up to 2,900 m in elevation and *I. tenuituba* at and above 3,200 m, along with a natural hybrid zone between the parent

ranges (Campbell et al. 1997). The wettest site is *I. agregata* population L, our lowest elevation site at 2,900 m (Campbell and Waser 2001). The driest site (I) is located in the middle of the hybrid zone, at 3,050 dm on a steep talus slope with little vegetation, population I (Campbell et al. 2004). Our highest elevation site is at *I. tenuituba* population A near 3225 m. There has been 23 years since the last time elevational cline data were collected from wild individuals, which is about 5 generations ago (Campbell 1997)

Trait measurements

To address my first question, at least two times per week, up to three open flowers from 10 plants were removed from each of the 12 populations, and the following traits were measured: corolla length, corolla width, anther position (using calipers), and 48-hr nectar production and sugar concentration (using capillary tubes and a refractometer as described in Campbell et al 1991). The majority of nectar measurements were collected on July 16th and 28th , with the remaining measurements collected August 4th. Corolla length and width were chosen to compare data from 1991 and 1992. However all of these traits can influence pollination success (Campbell et al. 1997)

SLA measurements

Specific leaf area (SLA) is the one-sided area of a fresh leaf divided by its oven-dry mass. Specific leaf area was calculated by collecting one leaf from each plant along our transects at each of the 12 sites between July 16th and July 22nd.

The area of each leaf was calculated by scanning the leaf using the program ImageJ. Samples were then oven dried and massed individually, and the mass was then used to calculate SLA (as area (cm^2) / dry mass (g)). These values were also averaged for each site.

Common Gardens

In 2007 seeds produced by hand-pollinated crosses of *I. aggregata* and *I. tenuituba* (parental, F_1 reciprocal hybrids, and F_2 hybrids) were planted randomly at 10 cm intervals within 20 1×1 m blocks at each of the three experimental sites: *I. aggregata* population L, hybrid population I, and *I. tenuituba* population C. Blocks were placed along strips where there were no flowering *Ipomopsis* individuals from the natural population within 1 m to avoid encroachment of wild seeds. In 2008, 10 blocks of F_2 hybrids were planted at the lowest experimental site to increase sample size. For this study, F_2 hybrids provide a large variation in trait values for selection to act upon. Each June, the surviving experimental plants are censused. Longest leaf length and number of leaves are measured for plants lacking inflorescences at that time. *Ipomopsis* exist as vegetative rosettes for a median of 5 years (at the lowest experimental site), bloom during a single season, set seed and die (Campbell 1997).

Data Analysis

I. Comparison of clines in 1992 vs 2015

To evaluate question 1, an analysis of covariance was used to investigate floral traits data; corolla length, corolla width, and anther position; comparing fit

to historical data, collected in 1992, with the data collected in 2015, with year as a factor and distance along the elevational transect as the continuous variable. Distance along the cline was estimated from data in Wu and Campbell (2005) and should ultimately be corrected using the 2015 GPS information.

II. Comparison of clines across traits in 2015

To compare clines in different traits, population means were scaled between zero and one by using:

(Y-min)/(max-min), where Y is the population mean, and min and max are the overall minimum and maximum population means.

These scaled values were then plotted against distance along the transect, for each trait in the direction that values increased with distance (Fig. 2). The best fitting polynomial function to each cline was used to describe cline variation. A liner regression was used to assess the SLA cline.

III. *Common Gardens*

To evaluate question 2, an analysis of variance on floral traits was conducted using site (e.g. I. aggregata, I tenuituba, or hybrid site), year, and plant genotype of origin (*I. aggregata, I tenuituba*, F1 hybrid AT, the reciprocal F1 hybrid TA, or F2 hybrid) as categorical predictors. All interactions were considered within the model, as based on previous work the responses of plants are expected to vary by genotype (*Ipomopsis aggregata*, *Ipomopsis tenuituba,* and cross-species hybrids) at a given site (Campbell, 2003; Campbell et al., 2010). A site effect would indicate plasticity, whereas an effect of genotype of origin

would indicate a genetic effect. An interaction would indicate a genotype by environment interaction.

Results

I. Comparison of clines in 1992 vs 2015

Neither corolla length nor corolla width showed a year by distance interaction in analysis of covariance (P = 0.636 and 0.2235). In a standard analysis of covariance with a factor of year and distance as a continuous variable, both traits were larger on average in 2015 than in 1992 (Fig. 1). Corolla length was larger by 4.4 mm in 2015 (Fig. 1A, $F_{1,242} = 13.54$, P = 0.0003), and corolla width was larger by 0.11 mm (Fig. 1B, $F_{1,242} = 7.83$, P = 0.0055). To evaluate whether that increased flower size was systematic over years, we also examined data from 1991 (all populations), 1999 (populations I and L only) and 2013 (populations I and L only). Corolla length did not differ significantly between 1991 and 1992 (ANCOVA on population means, $F_{1,20} = 0.09$, P = 0.7725), and that trait appeared to increase systematically over years (note the consistent difference between open symbols and filled symbols in Fig. 1A). On the other hand, corolla width was variable from year to year and did not show a systematic difference (Fig. 1B).

The highest anther position showed a more complex pattern, in which the difference between 1992 and 2015 depended upon the location on the transect (year x distance interaction, $F_{1,241} = 10.69$, P = 0.0012). That trait changed the most for *I. aggregata* populations lowest on the mountain (those with the largest values for distance in Fig. 1C), while changing little at the high *I. tenuituba*

populations. A strong clinal pattern with lower anther positions at the *I. aggregata* populations had been seen in 1992 (P = 0.0001 in model with distance effect nested within year), but no evident cline was seen in 2015 (P = 0.2142). Anther position did not differ significantly between 1991 and 1992, $F_{1,20}$ = 1.12, P = 0.3022). Thus the large values in 2015 are not just an anomaly.

II. Comparison of clines across traits in 2015

The slope for SLA was slightly lower than for the floral traits of corolla length, width, and 48 hour nectar production (Fig. 2). However, this vegetative trait did show a significant change with distance (linear regression, $F_{1,127}$ = 6.32, P = 0.0132). Site K had the highest SLA value, 202.774 cm^2/g, while site F showed the lowest SLA value, 145.273 cm^2/g (Table 1). The lowest elevation site, L, had the greatest 48 nectar production, 4.88 μL, while site B had the lowest 48hr nectar production with less than a microliter, 0.25 μL.

Table 1: Nectar Volume and Specific Leaf Area

	48 hr Nectar Production		Specific Leaf Area	
Site	Mean Nectar μL	Standard Error	Mean cm^2/g	Standard Error
A	0.875	0.224478562	160.075	6.2449
B	0.25390625	0.12333331	157.735	7.7208
C	0.422794118	0.089833776	148.826	6.2414
D	0.637019231	0.126586569	153.67	13.0237
E	0.572916667	0.107897595	173.909	28.1817
F	0.625	0.166521928	145.273	7.2015
G	1.171875	0.274440126	159.207	9.1548
H	0.846354167	0.308781863	170.533	15.0335
I	1.960227273	0.392808277	162.32	33.4459
J	3.701171875	0.382163919	148.829	10.2819

K	4.308035714	0.920799995	202.774	12.4115
L	4.876077586	0.44783913	178.849	5.6995

III. Common Gardens

Since both corolla length and highest anther position showed changes between 1992 and 2015, we analyzed whether those traits show genetic or environmental variation. In the common garden experiment, corolla length depended on both type of plant (a*ggregata*, F1 hybrid with *aggregata* mother, F1 hybrid with *tenuituba* mother, F2, or *tenuituba*; $F_{4,152} = 7.22$, $P < 0.0001$) and on the site of planting ($F_{2,152} = 4.94$, $P = 0.0084$). Those results indicate both a genetic effect and an environmental effect on the trait. Anther position also showed both genetic and environmental components of variation ($P = 0.0115$ and $P = 0.0345$ respectively).

Discussion

We have demonstrated that corolla length in Poverty Gulch, Gunnison County *Ipomopsis* has a significant genetic and environmental basis. Since length has a genetic component and it changed systematically over years, that change is consistent with an evolutionary difference when compared to the same populations from 23 years ago. However, alternative hypotheses need to be considered. Soil moisture has been shown to correlate with increased corolla length and may have influenced 2015 floral trait measurements (Campbell 2013). Additional data from a future dry year would help to distinguish these possibilities.

Although a significant genetic difference has been found in both corolla length and width, corolla width did not establish system wide variation when supplementary data from additional years were compared. Additionally, anther position varied significantly only when comparing lower elevational sites between 1992 to 2015. That change is consistent either with introgression of genes from the high elevational *I. tenuituba* sites or with environmental differences just at low elevation.

Trait clines in this study were evaluated by analyses of covariance. Yet, clines could be assessed in more depth by fitting clines to sigmoidal functions determined by the width, center position and maximum slope, and comparing cline parameter values as in Derryberry et al. (2014).

When considering snowmelt date as a means to infer soil moisture, one would speculate that a later snowmelt date would suggest higher soil moisture. Years with a later snowmelt date would have a systematic shift in higher soil moisture and in turn should have longer flowers across all population. With this logic longer flowers would be measured in 1999 (May 25th), 1991 (May 22nd), and 2013 (May 15th) compared to snowmelt date in 2015 (May 7th) and we do not see that occurring (http://www.gothicwx.org/). There was no significant difference in corolla length when comparing flowers from 1991 and 1992, with snowmelt dates of May 3rd and May 22nd respectively (http://www.gothicwx.org/). The changes in corolla length between years lesslikely to correlate with water moisture or seasonal snowmelt date and more likely to represent genetic changes.

Two possible explanations for this genetic variability are based on (1) pollinator selection or (2) a moving hybrid zone. Pollinator selection as a means

for our observed increased corolla length has the most supporting evidence, because of the systematic shift in corolla length across all populations along the elevational cline. Pollinators responsible for this selection include the hawk moth *Hyles lineata*, the Rufous Hummingbird (Selasphorus rufus*)*, and the Broad-tailed Hummingbird (*Selasphorus platycercus*). Both Hummingbirds and Hawk moths prefer to visit longer flowers (Campbell et al 1997, Campbell et al 1991). The alternative hypothesis, moving hybrid zone, suggests that longer corolla length genes are moving down the elevational cline through increased hybridization. Evidence for this hypothesis occurs when a trait remains the same in one section of a cline and has a significant difference in an adjacent section, shown in the anther position cline. This hypothesis is unlikely to be true, because this pattern is only shown in one floral trait.

Hybrid zone movement in the present and recent past has been found to be a wide spread occurrence (Buggs 2007). Patterns in natural zones are complex and have been found to be misleading, where phenotypic characters can show introgression through hybrid zones in different directions (Buggs 2007). Similarly, *Ipomopsis* floral trait patterns are not systematically uniform and show evidence of possible introgression in different directions.

Although evidence for environmental and evolutionary pressures selecting for floral trait differences along a hybrid zone was found, hypotheses explaining these movements remain untested. The preliminary hypothesis of global warming has been suggested as a cause of the movement of hybrid zones (Buggs 2007). However, the need for more hybrid movement studies is essential. Evidence of common trends need to be found in multiple hybrid systems before

climate change can be accredited for a causative factor hybrid zone movements (Buggs 2007).

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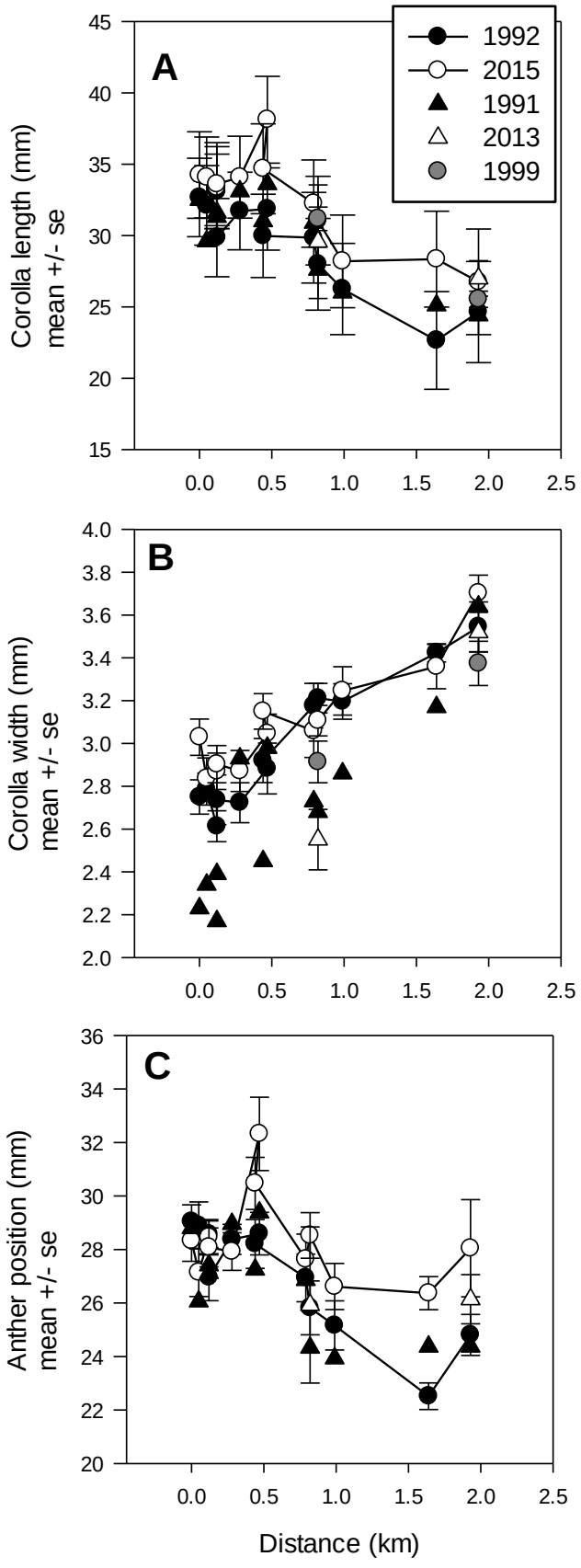
A
B
C
1992
2015
1991
2013
1999
Corolla length (mm)
mean +/- se
Corolla width (mm)
mean +/- se
Anther position (mm)
mean +/- se
Distance (km)

Fig. 1: Temporal and Spatial Floral Trait Variation
Means and standard errors across elevational clines are shown, where large black circles represent past data and open circles represent data from 2015. Ancova shows a significant 2015 Corolla Length increase over values in 1992 (P= 2.38e^-4).

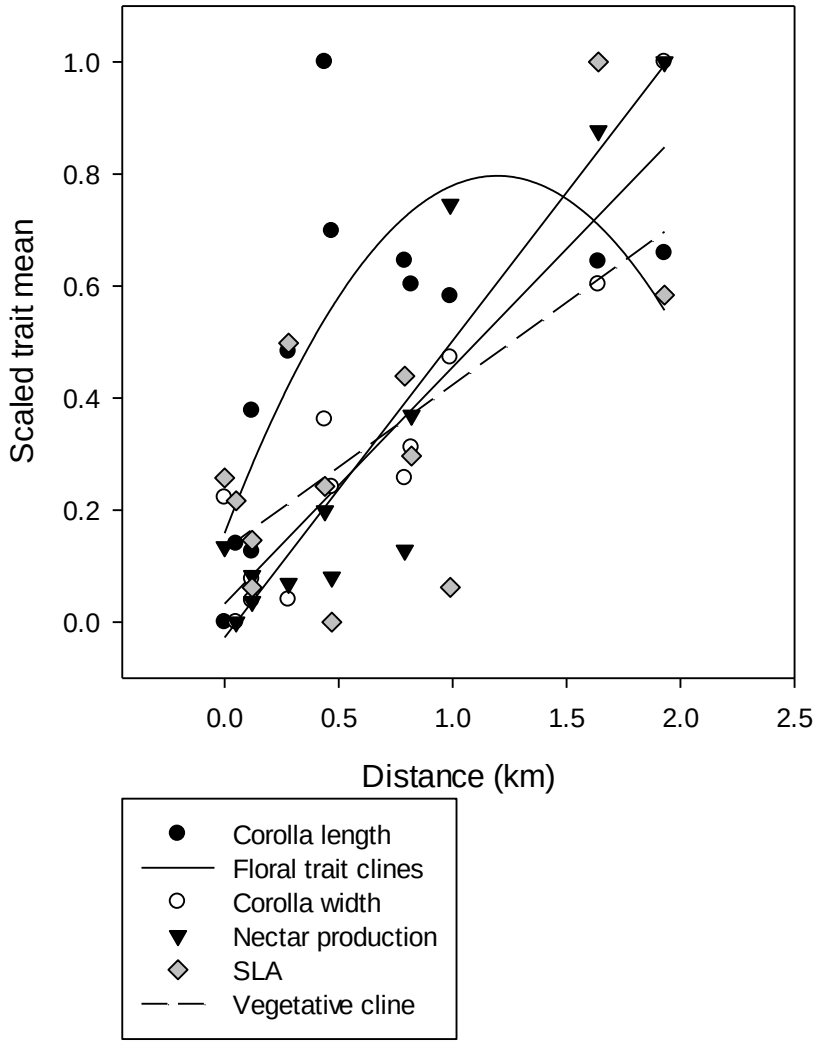

Fig. 2: 2015 Floral and Vegetative Clines

To compare clines in different traits, population means were scaled between zero and one by using: (Y-min)/(max-min), where Y is the population mean. Circular points represent corolla traits, triangular points represent 48 hour nectar production, and rhomboidal points present specific leaf area.

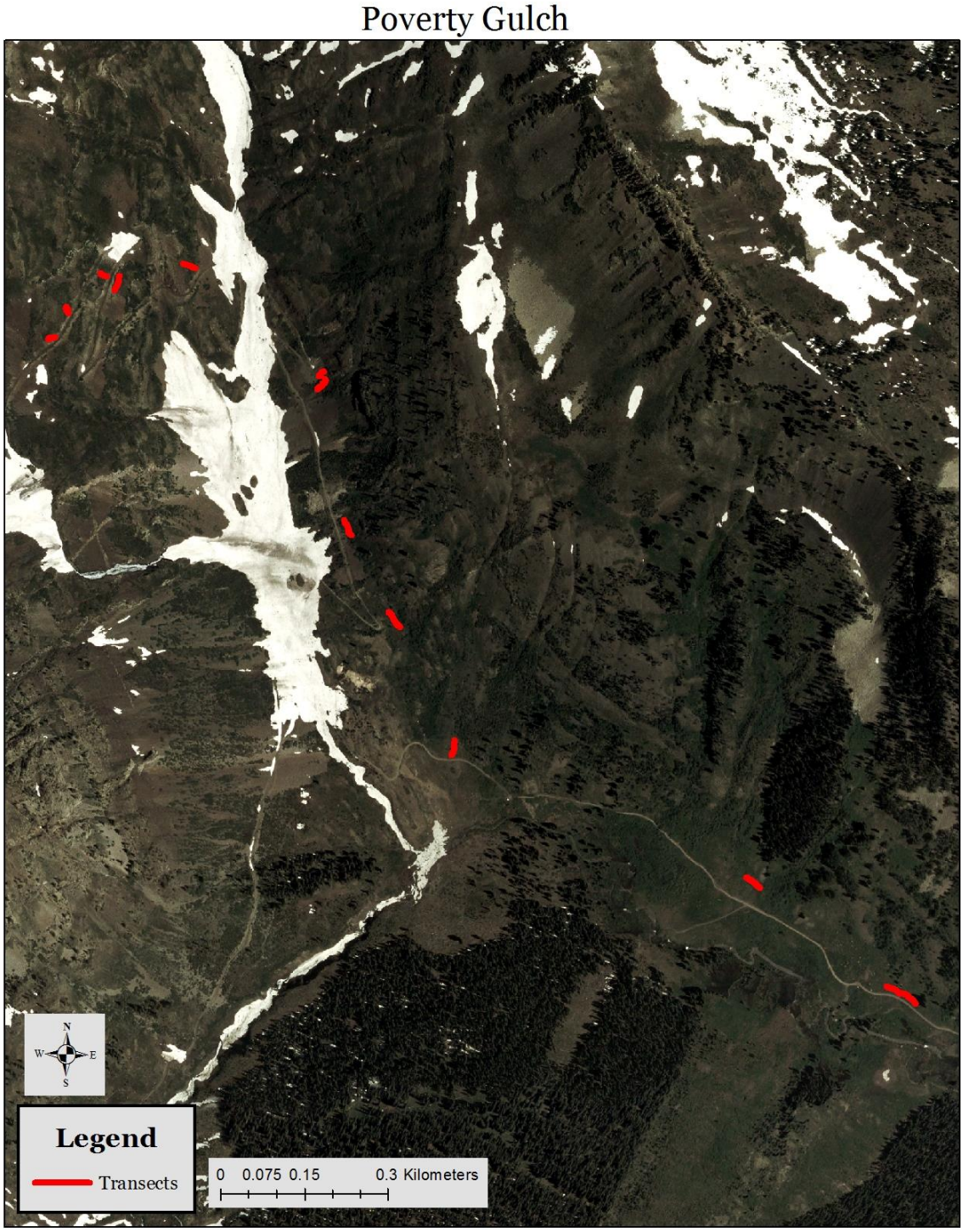

Fig. 3: Poverty Gulch Map
Population sites indicated by red transects.

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