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**Effects of age, density, and seasonality on molt pattern in the  
mammal genus (*Peromyscus*)**

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Honors Thesis, 2015

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## Abstract

Molting, or replacement of pelage (hair) in mammals, occurs during ontogeny as individuals transition from juveniles to adults. Environmental factors can cause variation in molt in many species for thermoregulatory and camouflage purposes. Within and among years molt can vary by time and age or by reproductive status. Past studies have found differences in molt pattern and timing depending upon age, and between captive reared and wild caught individuals. There has been little investigation into the molting characteristics of *Peromyscus leucopus* and *P. maniculatus*, specifically in comparison to adults and juveniles. I used molt observed from museum specimens collected at Bartlett Experimental Forest over a three-year period to determine if there are evident molt patterns in both *Peromyscus* spp. Specifically, I looked for differences in percent molt and number of individuals molting as a result of seasonality and age. Molts were digitized in Photoshop and pattern and symmetry were analyzed in ImageJ. In both species, individuals show a seasonal trend in molt timing and symmetry. This may reflect differences in resource availability and energy expenditure among years.

## Introduction

Many species have the capacity to molt. Molting is essentially the shedding of old or worn down exoskeleton, pelage, or skin that will be replaced by new growth. In mammals, molting of pelage (or hair and fur) can occur for different reasons including ontogenetic, thermoregulation, or camouflage. Ontogenetic molt and color change, as juvenile individuals transition to adulthood is common to many mammals. Mice in the genus *Peromyscus* undergo an ontogenetic molt characterized by juveniles with a gray pelage that molt to a tawny or “cinnamon red” pelage as adults (Collins 1923, Gottschang 1956, Brown 1963). Molting that occurs for thermoregulation is centralized around the thickness and length of hair (Tabacaru *et al.* 2014). During the winter months, the density and thickness of hair is greater to retain heat and maintain a functional body temperature. In the summer season, pelage is generally thinner and less dense so that individuals do not overheat. In addition to ontogenetic and thermoregulatory molts, predator avoidance may trigger molting which results in camouflage. For example, Ermines (*Mustela erminea*) and Snowshoe hares (*Lepus americanus*) will transition from a white pelage in the winter, to a brown pelage in the summer.

Molting can be affected by both environmental and physiological factors. Specifically, these factors can affect the occurrence and timing of molt as well as the amount, consistency, and spatial patterning of molt. Age, energy expenditure, and resource availability are the key factors that affect molt. As previously mentioned, ontogenetic molt with age (juvenile to adult) is common in small mammals. Not only does this molt often include a change in pelage color but; past studies have found that there is a consistent and reliable pattern in this molt (Gottschang, 1956). Energy expenditure also significantly affects molt. Molting is an energetically expensive adaptation and therefore it is not believed to occur at the same time as other energetically expensive life processes such as reproduction (Tabacaru *et al.* 2014). Similarly, molting will require reliable and adequate resources to sustain an individual during this process. When resources are limited, an

individual may not molt or the molt will consist of patchy and inconsistent pelage regrowth (Tabacaru *et al.* 2014 and Sare *et al.* 2005).

In this study, the molts of both the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*) are investigated. These species are generalists in terms of diet and habitat and therefore are abundant and have a wide distribution. *Peromyscus leucopus* can be found from Canada to Mexico and as far west in the United States as Arizona. *Peromyscus maniculatus* can be found from Canada to Mexico as well and are found in every state in the United States except Florida (Hall, 1977). Past studies on molt in *Peromyscus* have focused on captive raised individuals and have had relatively small sample sizes. These studies have also focused specifically on adults or juveniles or male individuals (e.g., Gottschang, 1956 and Tabacaru *et al.* 2014). Here, we use wild caught individuals and a large sample size encompassing males, females, juveniles and adults.

We focus on quantifying the spatial pattern of molting individuals. The percent molt, or the area of the individual molting, is measured and the symmetry of dorsal and ventral molts of an individual is correlated. The results from these analyses can be a good indicator that physiological and environmental factors may be affecting the molting of pelage. In addition to using these analyses to determine if there is evident symmetry in the molts of both *Peromyscus* species, we investigate if the number of individuals molting in a population varies over the summer season as well as if the percent of an individual molting varies over the summer season. We test whether percent molt and symmetry of molt are affected by age and reproductive status.

## Methods

The mice were collected from nine sites at the Bartlett Experimental Forest in the White Mountains of New Hampshire in the summers of 2012, 2013 and 2014. Both *P. leucopus* (n= 240) and *P. maniculatus* (n=164) had large sample sizes. The individuals were given to us courtesy of a long-term monitoring program conducted by the U.S. Forest Service. The individuals were then prepared as museum specimens. During this process, standard external measurements, weight, and reproductive condition were noted for each individual. The age of each individual was determined by weight. For *P. leucopus*, a larger species, individuals that weighed 19.0 grams or more were considered adults. For *P. maniculatus*, individuals that weighed greater than 16.0 grams were considered adults in this study. Reproductive status was determined for both males and females. Reproductively active males were those whose testes mass was 7.0 grams or greater. Reproductively active females were those individuals that showed evidence of lactation, or had embryos present during preparation.

Preparation of study skins provides opportunity to record the pigment pooled in the hair follicle that can be seen from the underside of the skin. This pigment is an indicator of the molting that is occurring in the individual. The pigment was drawn onto molt sheets for the dorsal, ventral and lateral sides of each individual. The molt sheets were then scanned and digitized in Photoshop. ImageJ was then used to quantify the percent molt of the dorsal and ventral sides of each individual by measuring the pixels of the digitized molt drawings. The total percent molt of the individual was calculated by adding the percentages of the dorsal and ventral molts. ImageJ was also used to quantify the symmetry of dorsal and

ventral molt drawings by overlaying the left and right sides of a designated molt and calculating the correlation between the images.

For each year (2012, 2013, and 2014) the number of molting adults and juveniles in a population were analyzed over the summer months of July, August, and September. T-tests were used to test for significance between juveniles and adults in the designated summer months for average dorsal symmetry, percent molt, and reproductive status with a p-value of 0.05 being the indicator of a significant difference or not. Sample numbers of individuals from both *Peromyscus* species were combined during assessment of percent of individuals molting, average dorsal symmetry, average percent molt, and reproductive status. Data from 2012, 2013, and 2014 was combined to assess average dorsal symmetry, average percent molt, and reproductive status, but not percent of individuals molting.

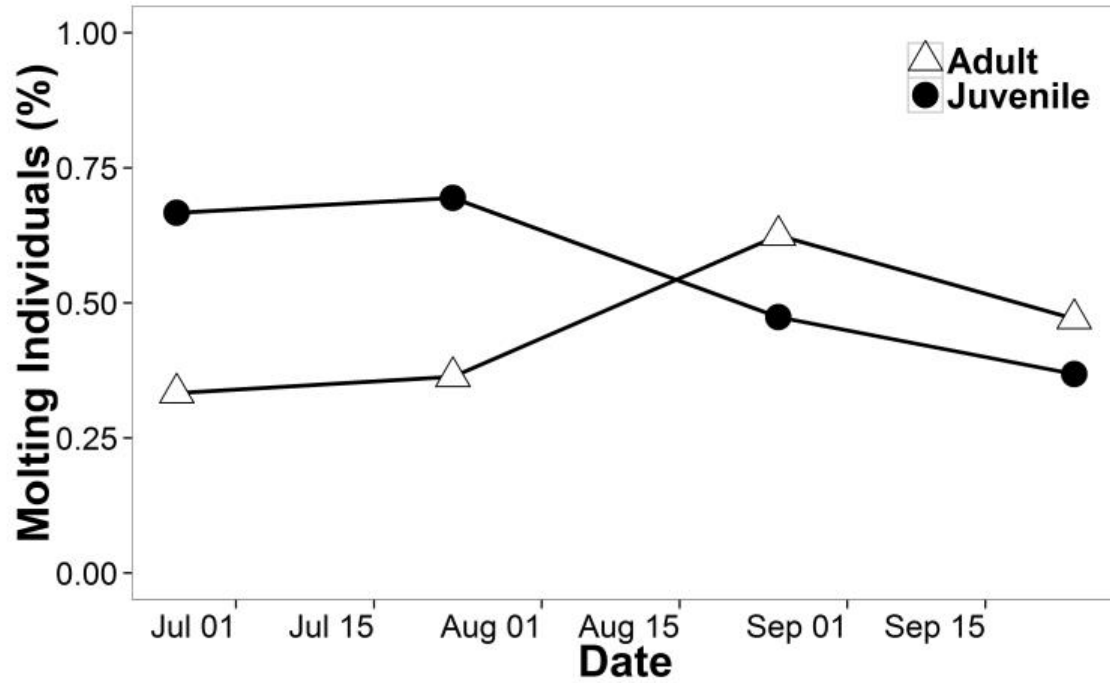
## Results

Timing of molt varied among juveniles and adults. For 2014, juveniles have a higher number of molting individuals in the early summer months (July and early August) while in late August and September the number of molting individuals decreases (Figure 1). There is an opposite trend in the adults as the number of molting individuals is low in early in the season and increases in late August and September (Figure 1).

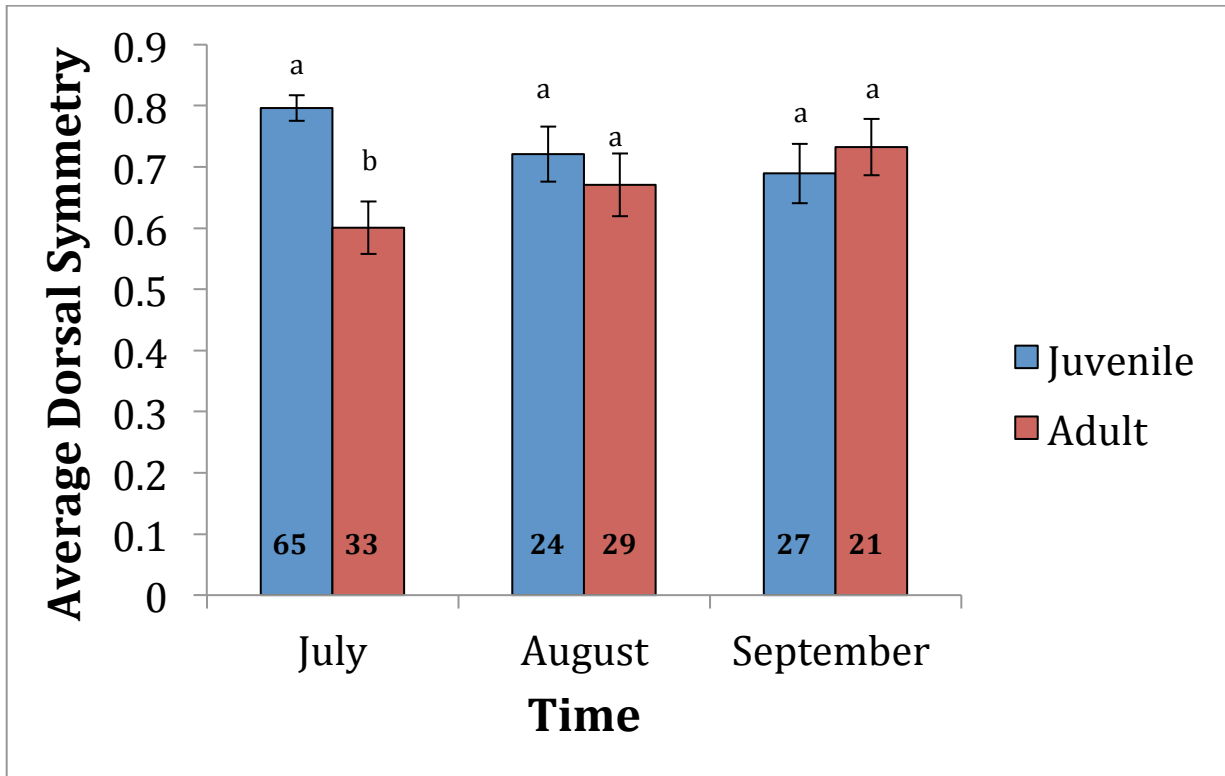
The average dorsal symmetry of the molts of juveniles and adults was assessed over July, August, and September. There is significant difference between the dorsal symmetry of juveniles and adults in July ( $p= 0.000267$ ) but not in August ( $p= 0.439$ ) and September ( $p=0.687$ ) (Figure 2). There is a gradual decrease in dorsal symmetry over the three months for juveniles but a gradual increase in dorsal symmetry for adults.

The average percent molt, or area of an individual molting over July, August, and September is shown in Figure 3. In July, there is a significant difference between area of juveniles molting and area of adults molting ( $p= 0.000456$ ). In both August ( $p= 0.584$ ) and September ( $p=0.871$ ) there is a not a significant difference between area of molt in juveniles and adults.

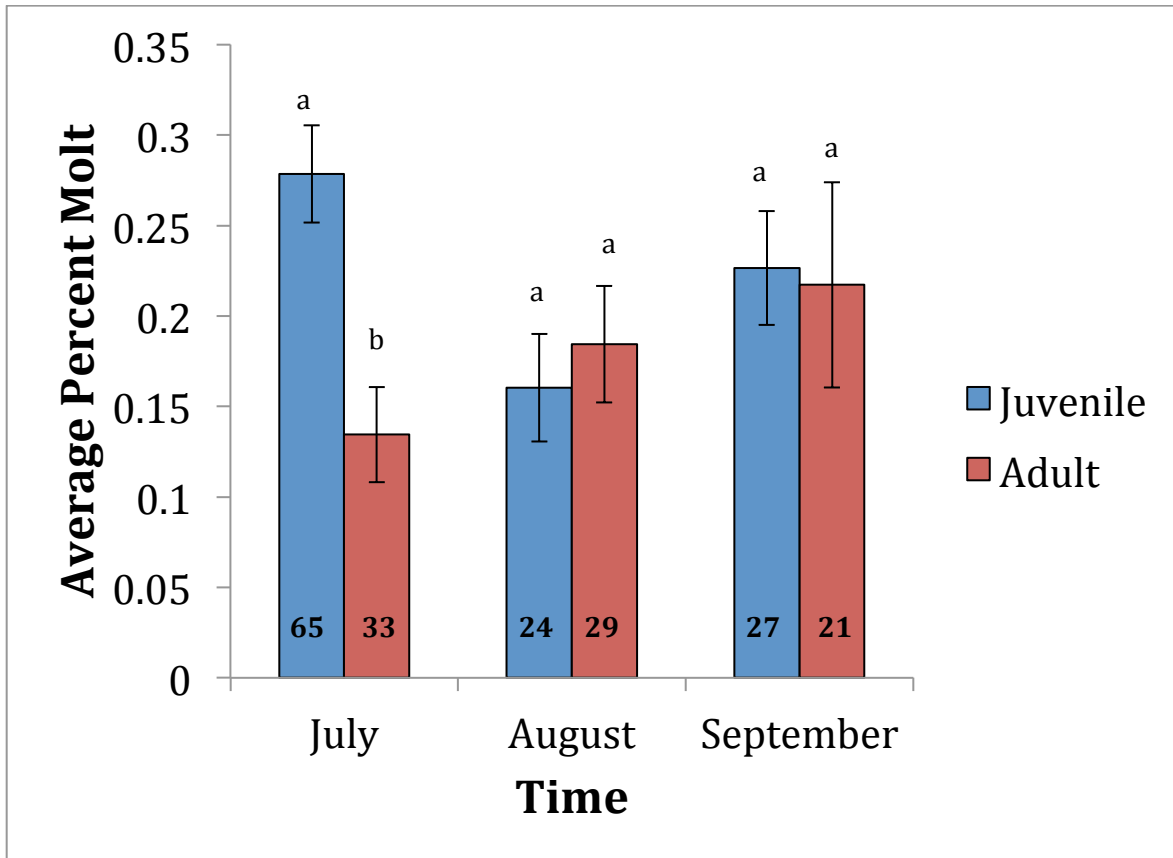
Effect of reproductive status on molt was tested for both males and females. Average percent molt of an individual versus reproductive status (non-breeding and breeding females and males) did not differ between non-breeding individuals and breeding individuals for males ( $p= 0.463$ ) or females ( $p= 0.825$ ). It is worth noting that there is a small sample size ( $n=7$ ) of breeding females; however, males have a large sample size for both breeding and non-breeding individuals and demonstrate similar results.



**Figure 1** Graph of percent of individuals molting in a population over July, August, and September in the year 2014. Number of juveniles molting decreases over the summer. Number of adults molting increases over the summer.

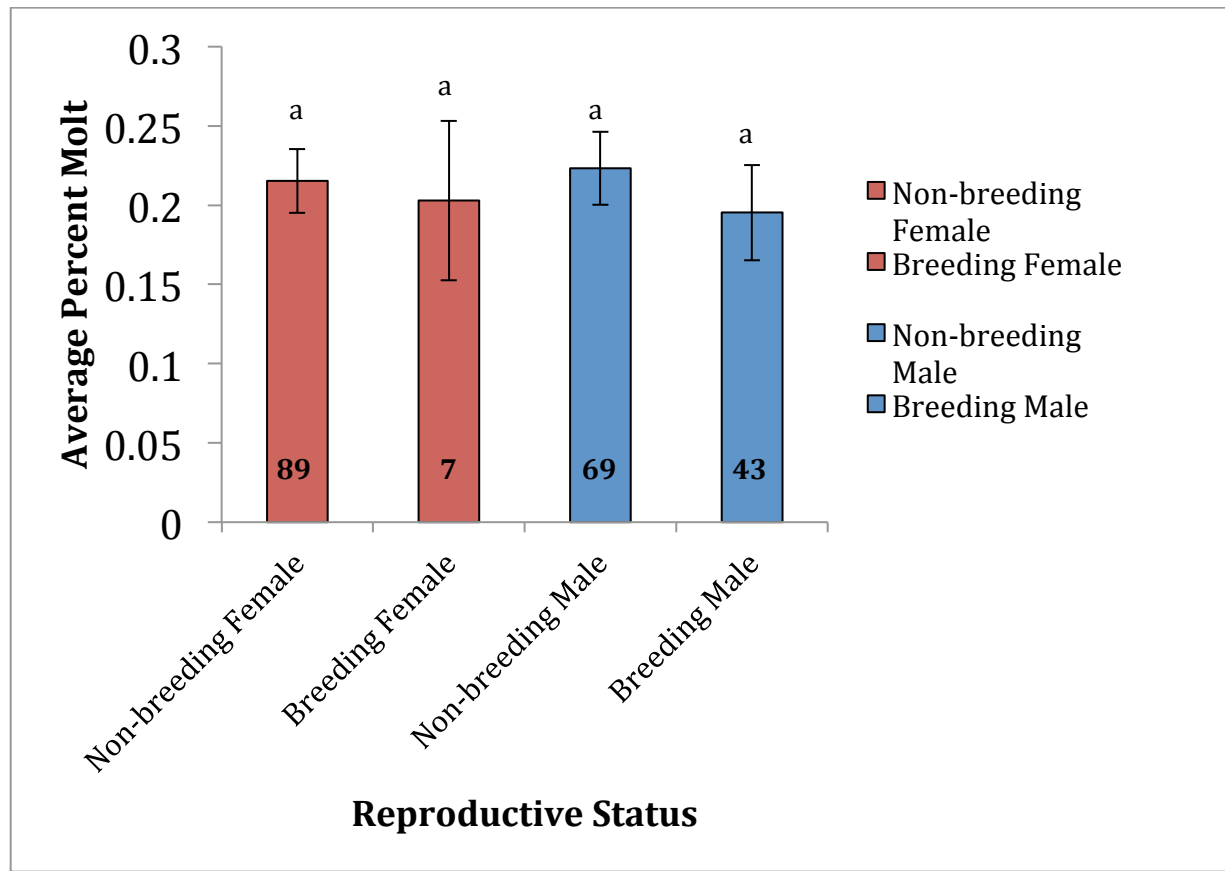


**Figure 2** This graph shows the average dorsal symmetry (correlation coefficient) of the molts of juveniles and adults over a three-month period for years 2012-2014 combined. Trends in dorsal symmetry can be seen in both adults and juveniles. The data from 2012, 2013 and 2014 was combined to assess average dorsal symmetry. Different letters above the bars indicate a significant difference ( $p < 0.05$ ) between the juvenile and the adult values per month. The sample size of each group is also given in the bottom of each bar.



**Figure 3** Graph of the average percent molt of both juvenile and adult *Peromyscus* over a three-month period across sampling years. Molting trends can be seen in both juveniles and adults. The data from 2012, 2013 and 2014 was combined to assess average percent molt. Different letters above the bars indicate a significant difference ( $p < 0.05$ ) between the juvenile and the adult values within a month. Sample sizes are given in the bottom of each bar.





**Figure 4** The average percent molt of an individual in regards to sex and reproductive status. No significant differences were detected between sex and reproductive status in terms of percent molt. The data from 2012, 2013 and 2014 was combined to assess reproductive status.

## Discussion

The process of molting is affected by many factors, both environmental and physiological. This study focused on reproductive status, age, and seasonality. In the early season of July, the increase in juvenile percent molt and average dorsal symmetry are most likely a function of ontogenetic development. The peak-breeding season for *Peromyscus* is in late May and early June. Juveniles tend to retain their gray pelage for about 40 days before they start their transitional adulthood molt (Gottschang, 1956). July would correspond with the timing of this transition and could explain why there is a greater percent of individuals molting, a greater average percent of the area of an individual molting, and greater average dorsal symmetry in juveniles. Average adult percent molt and average dorsal symmetry are lower in the month of July compared to juveniles. This could be because of two reasons that are distinguishable between males and females. These adults will have just ended their peak-breeding season. For males, the physiological stress of trying to acquire mates and breed can often vary the time these individuals spend foraging and can limit their resource intake, which can lead to less, more inconsistent molt (Tabacaru *et al.* 2014 and Sare *et al.* 2005). On the other hand, reproduction is very

energetically expensive for females that must not only carry their young prior to birth, but must provide for them after birth in the form of lactation, another physiologically expensive process (Tabacaru *et al.* 2014). Without proper energy expenditure dedicated to molt these individuals would most likely have inconsistent and limited molt as well.

During August and September, the increase in number of individuals molting and the similarity between juvenile and adult molt could be explained by seasonality and for juveniles, timing of birth. At this point, the summer season is ending and the colder winter season is approaching. Adults are now starting to molt and transition into their winter pelage that is thicker for thermoregulation purposes (Tabacaru *et al.* 2014 and Sare *et al.* 2005). This seasonal transition molt would cause the amount of adults that are molting to increase and would also increase the average percent or area of the individual molting and the average dorsal symmetry of the molt. While late May through early June is known to be the peak-breeding season for *Peromyscus*, a later breeding period can occur mid-summer. These later born individuals are just now undergoing their ontogenetic molt; which could explain the similarity in symmetry and percent molt of juveniles to adults. .

Past studies have stated that molt will not occur during times of other energetically expensive life processes such as reproduction (Tabacaru *et al.* 2014 and Sare *et al.* 2005). However, my results suggest breeding does not influence molt in both female and male individuals of *P. leucopus* and *P. maniculatus*. For females, small sample size may have contributed to the non-significant difference in area of percent molt between breeding and non-breeding individuals. Although we did have a larger sample size of breeding males, we also detected no significant difference between the percent molt of breeding and non-breeding males. In addition, these individuals were collected during the later/summer breeding period (July and early August) and not the peak-breeding season (late May-early June), which could have skewed the results in terms of percent molt in breeding individuals. Our results can also be explained by a requisite winter molt. Whether the individuals we collected were breeding in the later season or not, they must begin their seasonal transition to winter pelage in order to survive the winter months. This would also explain the similarity in percent molt of breeding and non-breeding individuals.

Molting of pelage is an extremely variable process. As previously mentioned, there are many factors that can affect molt. The average dorsal symmetry and percent molt of the studied individuals indicate that both environmental and physiological factors do have implications on molt in terms of seasonal transition and ontogenetic development. In the future it would be interesting to attain individuals from the peak-breeding season and determine if in fact individuals truly restrict their molt during such an energetically expensive time. In order to get a more accurate timing of variation in molt between juveniles and adults, aging individuals by tooth wear can give a more precise understanding of trends in molt over time. In addition there is potential for molt to be used to better understand diet. Stable isotope analysis on hair provides an integrated signal of an individual's diet spanning from a few weeks to a year. Sampling hair following known molting episodes may help isolate the contribution of food items to diet during discrete time periods. Comparing molt to isotopic analysis of hair can create ideas about trophic relationships.

## Acknowledgements

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