UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGÍA

BIOLOGÍA EVOLUTIVA

UN VÍNCULO ENTRE EL ENVEJECIMIENTO Y LA REPRODUCCIÓN EN LOS PERROS APOYA LA TEORÍA DEL ENVEJECIMIENTO DEL SOMA DESECHABLE

TESIS

(**POR ARTÍCULO CIENTÍFICO**)

A LINK BETWEEN AGING AND REPRODUCTION IN DOGS SUPPORTS THE DISPOSABLE SOMA THEORY OF AGING

QUE PARA OPTAR POR EL GRADO DE:

MAESTRO EN CIENCIAS BIOLÓGICAS

PRESENTA:

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CD. MX. JUNIO, 2021

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P r e s e n t e

Me permito informar a usted que en la reunión ordinaria Comité Académico del Posgrado en Ciencias Biológicas, del día 26 de abril de 2021,se aprobó el siguiente jurado para la presentación del examen para obtener el grado de **MAESTRO EN CIENCIAS BIOLÓGICAS** en el campo de conocimiento de **(Biologìa Evolutiva)** del estudiante **BARGAS GALARRAGA IKER** con número de cuenta: **309558741**, en la modalidad de graduación de **tesis por artículo científico** titulado: "**A link between aging and reproduction in dogs supports the disposable soma theory of aging",** que es producto del proyecto realizado en la maestría que lleva por título: **"UN VÍNCULO ENTRE EL ENVEJECIMIENTO Y LA REPRODUCCIÓN EN LOS PERROS APOYA LA TEORÍA DEL SOMA DESECHABLE",** Bajo la dirección del **DR. ALEJANDRO EMMANUEL GONZÁLEZ VOYER,** quedando integrado de la siguiente manera:

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Secretario:	DR. JOSÉ JAIME ZÚÑIGA VEGA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 07 de junio de 2021

COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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Índice

Resumen

Las prominentes diferencias en el envejecimiento entre especies y dentro de ellas presentan un rompecabezas evolutivo. Las teorías propuestas para explicar las diferencias evolutivas en el envejecimiento se basan en el axioma de que la selección maximiza la adecuación, no necesariamente la longevidad. Esto implica disyuntivas entre el mantenimiento somático y la inversión en el crecimiento y la reproducción, donde un crecimiento rápido y alta inversión en la reproducción a corto plazo se asocian con una longevidad corta. Sabemos que un crecimiento más rápido está relacionado con una longevidad más corta en el perro doméstico, un modelo novedoso en la investigación del envejecimiento, sin embargo, desconocemos si la reproducción influye en la longevidad. Aquí analicé si la reproducción está relacionada con las diferencias en la longevidad entre las razas de perros, controlando simultáneamente por la ascendencia compartida y el flujo génico entre razas de perros. Los resultados muestran que la inversión reproductiva tiene un impacto negativo sobre la longevidad, sin embargo la magnitud del efecto depende del tamaño de los adultos, siendo las razas grandes las que pagan un costo en longevidad mayor; esto indica que el efecto no es simplemente una respuesta correlacionada del tamaño del adulto. Estos resultados concuerdan con las predicciones de la teoría del envejecimiento del soma desechable, lo que sugiere que las diferencias entre razas en la longevidad se deben a una combinación de tamaño (potencialmente crecimiento) y reproducción.

Palabras clave: envejecimiento, reproducción, crecimiento, disyuntiva, perro doméstico

Abstract

Prominent differences in aging among and within species present an evolutionary puzzle. The theories proposed to explain evolutionary differences in aging are based on the axiom that selection maximizes fitness, not necessarily lifespan. This implies trade-offs between investment into self-maintenance and investment into reproduction, where fast growth and high investment into current reproduction are associated with short lifespans. Fast growth and large adult size have been found to be related with shorter lifespans in the domestic dog, a bourgeoning model in aging research, however whether reproduction influences lifespan remains unknown. Here we test the relationship between reproduction and differences in lifespan among dog breeds, controlling simultaneously for shared ancestry and recent gene flow. Our results show that reproductive investment negatively impacts lifespan, and more strongly so in large breeds, an effect that is not merely a correlated response of adult size. These results are in line with predictions from the disposable soma theory for the evolution of aging, suggesting that among-breed differences in lifespan are due to a combination of size (and potentially growth) and reproduction.

Key Words: Aging, reproduction, growth, trade-off, domestic dog

Introducción

El envejecimiento es el resultado del deterioro fisiológico de un organismo, lo que aumenta las probabilidades de morir. Entre los vertebrados hay diferencias notables en el envejecimiento, lo que resulta en una gran variación con respecto a la longevidad (Jones et al. 2014). Aunque el envejecimiento puede considerarse una generalidad, las diferencias prominentes entre especies componen un rompecabezas evolutivo. Las teorías propuestas para explicar las diferencias evolutivas en el envejecimiento se basan sólidamente en el axioma de que la selección maximiza la adecuación, no necesariamente la longevidad (revisado en Maklakov y Chapman 2019). Como resultado, la fuerza de la selección en un rasgo disminuye después de la maduración sexual y conforme avanza la edad, lo que resulta en la famosa "sombra de selección" de Haldane (Haldane 1941, Maklakov y Chapman 2019). Por lo tanto, la selección puede favorecer aquellos rasgos que otorgan beneficios en los primeros años de vida, incluso si estos mismos rasgos representan costos más adelante, especialmente si surgen después de la vida reproductiva. Estos efectos perjudiciales pueden ser consecuencia de efectos pleiotrópicos antagónicos, donde un mismo alelo tiene efectos beneficiosos en la vida temprana, pero efectos perjudiciales en la vejez (Williams 1957), o debido a disyuntivas evolutivas entre rasgos de historia de vida (Kirkwood 1977).

Debido a que los animales cuentan con una cantidad finita de energía, actividades energéticamente demandantes como el crecimiento o la reproducción inevitablemente consumen recursos que dejan de estar disponibles para otras actividades, como el mantenimiento somático (Kirkwood 1977; Chen et al. 2020). Como resultado, un crecimiento rápido y una alta inversión en la reproducción actual consumen recursos que no se pueden invertir en el mantenimiento somático, lo que resulta en un deterioro fisiológico más rápido (es decir, envejecimiento) y una menor longevidad (Kirkwood y Holliday 1979, Kirkwood y Rose 1991). Tales disyuntivas entre crecimiento, desarrollo, reproducción y mantenimiento somático conforman la base de la teoría de historia de vida (Kirkwood y Austad, 2000; Kaplan y Robson, 2009).

Existen evidencias entre especies y dentro de especies de que un crecimiento rápido compromete la longevidad de los individuos (Metcalfe y Monaghan 2003; Austad 2010). A nivel intraespecífico, ratones, ratas y perros seleccionados hacia una alta tasa de crecimiento o un mayor tamaño corporal presentan una menor longevidad (Patronek et al 1997; Miller et al. 2000; Bartke et al. 2001a; Rollo 2002). Sin embargo, algunas veces es difícil controlar efectos de confusión a nivel intraespecífico tales como las diferencias en la calidad de los individuos, el acceso a los recursos o simplemente el que la variación no sea suficiente como para detectar diferencias claras. A nivel interespecífico podemos observar diferencias más claras, por ejemplo, en general sabemos que las especies de crecimiento rápido muestran periodos de vida cortos, en comparación con las de crecimiento lento (Jones et al. 2008, Bielby et al. 2007). Sin embargo, en ocasiones tampoco podemos controlar otros efectos de confusión, tales como las diferencias en la mortalidad extrínseca, las cuales promueven diferentes estrategias de historia de vida que no necesariamente se relacionan a lo que queremos medir. Muchos estudios en vida silvestre están sujetos a medir las consecuencias de la reproducción en poblaciones sesgadas por dicha mortalidad extrínseca, fenómeno que enmascara la existencia de disyuntivas relacionadas con la longevidad. Una mayor comprensión de las disyuntivas que impulsan las diferencias en la longevidad requiere desenredar los efectos del crecimiento y la reproducción, idealmente en un entorno donde se minimicen variables de confusión tales como la mortalidad extrínseca y las diferencias individuales en la adquisición de recursos (van Noordwijk y de Jong 1986), en un modelo de estudio cuya variación entre rasgos de historia de vida sea suficientemente basta para contrastar.

El perro doméstico representa un modelo animal único, pues su biología contiene aspectos distintivos que son relevantes para los estudios del envejecimiento. Los perros y los humanos han coevolucionado y comparten procesos evolutivos de selección recientes, como la adaptación a la digestión de dietas ricas en almidón, además de que existen claros signos de evolución convergente en el genoma humano y canino (Theofanopoulou et al., 2017). Además, a diferencia de los animales de laboratorio, los perros tienen un entorno y estilos de vida muy similares

al del ser humano, y muchas veces están expuestos a los mismos contaminantes (Gilmore and Greer, 2015). También podríamos considerar que los perros están libres de mortalidad extrínseca, debido a la ausencia de depredadores, el acceso general a recursos suficientes y, a menudo, atención médica de calidad, sobre todo si consideramos únicamente perros de raza. Debido a la selección artificial, la variación entre razas de perros con respecto al tamaño corporal, longevidad y tamaños de camada es enorme. Los perros presentan un nivel extraordinario de variación fenotípica con respecto a su estructura esquelética, incluido el tamaño general, la longitud de las patas y las variantes de la forma del cráneo, incluso en comparación con todos los cánidos (Drake y Klingenberg, 2010). Estudios previos han documentado una asociación negativa entre la longevidad y el aumento del tamaño corporal en las razas de perros (Speakman et al. 2003; Fleming et al. 2011; Greer et al. 2011; Selman et al. 2013), al contrario de lo que se observa al comparar especies de mamíferos, pero de acuerdo con observaciones intraespecíficas (Patronek et al 1997; Miller et al. 2000; Bartke et al. 2001a; Rollo 2002). Sin embargo, no está claro si la selección del tamaño corporal y las consecuencias comúnmente correlacionadas con respecto a la inversión reproductiva, han dado como resultado la disyuntiva esperada entre reproducción y longevidad.

Las razas de perros presentan tanto un desafío analítico como una oportunidad. Muchas razas se han desarrollado principalmente durante los últimos cien años como resultado de la selección artificial, la separación geográfica, la migración y la hibridación (Parker et al.2004, Spady y Ostrander 2008). Existen más de 400 razas de perros descritas, de las cuales al menos 100 se suelen considerar como poblaciones estables, aisladas y controladas por las principales asociaciones canófilas. Se ha sugerido que el flujo génico juega un papel particularmente importante en la inmensa variación fenotípica observada entre razas (Parker et al. 2017). Aunque todas las razas de perros comparten un ancestro en común, entre ellas comparten diferentes grados de flujo génico. Esto implica que es poco probable que las relaciones entre las razas de perros puedan visualizarse mediante modelos evolutivos clásicos tales como árboles filogenéticos, pues técnicamente no existe una filogenia adecuada capaz de representar dichas relaciones de flujo génico. Por

lo tanto, los enfoques comparativos típicos que asumen la no independencia de las observaciones resultan inadecuados (Stone et al. 2011). Análisis genómicos relativamente recientes han permitido desenredar aquellas relaciones que corresponden al flujo génico más reciente, de aquellas que corresponden con el hecho de compartir una ancestría común relativamente más antigua, lo que nos permite revelar con mayor claridad la historia evolutiva de las razas de perros (Lindblad-Toh et al. 2005, Parker et al 2017; Garamszegi et al. 2020). Esta nueva información genómica también permite la realización de análisis capaces de considerar por separado los posibles efectos tanto de la ascendencia compartida como del flujo génico reciente, variables que hasta hace poco, al no poder considerarse por separado, incumplían con el supuesto de no-independencia de las observaciones necesario para la realización de modelos tradicionales (Felsenstein 2002; Stone et al. 2011).

Mi objetivo es analizar si la inversión reproductiva afecta la longevidad en las razas de perros, como predice la teoría del envejecimiento del soma desechable. Pienso que las razas con una alta inversión reproductiva (es decir, tamaño de camada multiplicado por peso de neonato), presentarán una menor longevidad. Utilicé un enfoque de modelos mixtos, con base en Garamszegi et al. (2020), que permite controlar simultáneamente la no independencia de las observaciones, con base en la ascendencia compartida y el flujo génico reciente (Felsenstein 2002, Stone et al.2011, Larson et al.2012, Parker et al.2017).

Objetivos

1. Analizar el efecto de la inversión reproductiva sobre la longevidad, considerando la influencia de la ascendencia común y el flujo génico a través de las razas de perros.

2. Analizar el efecto del tamaño de camada y el peso del adulto sobre la longevidad, considerando la influencia de la ascendencia común y el flujo génico, a través de las razas de perros.

3. Analizar el efecto de la inversión reproductiva y el crecimiento sobre la longevidad, considerando la influencia de la ascendencia común y el flujo génico, a través de las razas de perros.

Hipótesis y predicciones

La longevidad a través de las razas de perros estará asociada a diferencias en la inversión reproductiva, estimada por el tamaño de camada y el peso del neonato, de manera que:

1. La inversión reproductiva se relacionará de forma negativa con la longevidad, controlando por la ascendencia común y el flujo génico reciente, a través de las razas de perros.

2. El tamaño de camada se relacionará de forma negativa con la longevidad, tomando en cuenta el peso del adulto y controlando por la ascendencia compartida y el flujo génico a través de las razas de perros.

3. La inversión reproductiva se relacionará de manera negativa con la longevidad, tomando en cuenta la tasa de crecimiento y controlando por la ascendencia común y el flujo génico reciente, a través de las razas de perros.

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A link between aging and reproduction in dogs supports the disposable soma theory of aging.

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A link between aging and reproduction in dogs supports the disposable soma theory of aging.

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Abstract

Prominent differences in aging among and within species present an evolutionary puzzle. The theories proposed to explain evolutionary differences in aging are based on the axiom that selection maximizes fitness, not necessarily lifespan. This implies trade-offs between investment into self-maintenance and investment into reproduction, where fast growth and high investment into current reproduction are associated with short lifespans. Fast growth and large adult size have been found to be related with shorter lifespans in the domestic dog, a bourgeoning model in aging research, however whether reproduction influences lifespan remains unknown. Here we test the relationship between reproduction and differences in lifespan among dog breeds, controlling simultaneously for shared ancestry and recent geneflow. We found that shared ancestry explains a higher proportion of the among-breed variation in key life history traits, in comparison with recent gene flow. Our results also show that reproductive investment negatively impacts lifespan, and more strongly so in large breeds, an effect that is not merely a correlated response of adult size. These results are in line with predictions from the disposable soma theory for the evolution of aging, suggesting that among-breed differences in lifespan are due to a combination of size (and potentially growth) and reproduction.

Introduction

Aging is the result of physiological deterioration of an organism, which increases the probability of death. Across vertebrates there are striking differences in aging, resulting in very different lifespans (Jones et al. 2014). For instance, the Greenland shark (*Somniosus microcephalus*), lives more than 400 years and reaches sexual maturity at about 150 years (Nielsen et al. 2016). At the other extreme, the turquoise killifish (*Nothobranchius furzeri*) has a median lifespan of up to 7 months and can reach sexual maturity in 3-4 weeks in captivity (Kim et al. 2016). Although aging is ubiquitous, the prominent differences among species present an evolutionary puzzle. The theories proposed to explain evolutionary differences in aging are all solidly based on the axiom that selection maximizes fitness (i.e. survival and reproduction), not necessarily lifespan (reviewed in Maklakov and Chapman 2019). As a result, the strength of selection on a trait declines after sexual maturation and with advancing age, resulting in Haldane's famous 'selection shadow' (Haldane 1941, Maklakov and Chapman 2019). Selection can thus favor traits that bestow benefits in early life, even if these same traits incur costs later in life, particularly so if costs are only apparent towards the end of reproductive lifespan. Such late-life detrimental effects can result from antagonistic pleiotropic effects, where an allele has beneficial effects in early life, but has detrimental effects in late-life (Williams 1957), or due to functional trade-offs (e.g., if fast growth comprises lifespan; Kirkwood 1977).

Because animals have a limited energy budget, energetically demanding activities such as growth or reproduction, inevitably consume resources that will no longer be available for other energetically demanding activities, such as somatic maintenance, resulting in faster physiological deterioration and reduced longevity (Kirkwood 1977, Kirkwood and Holliday 1979, Kirkwood and Rose 1991, Chen et al. 2020). For example, under high adult mortality risk, a strategy favoring high reproductive investment early in life is favored even if it incurs costs in terms of reduced longevity, given the probability that the organism will not live to pay such costs. On the contrary, when adult mortality is low, selection favors partitioning investment into several reproductive events, allowing investment into somatic maintenance and prolonging lifespan (Jones et al. 2008; Healy et al. 2019). Such trade-offs between survival, growth, development, reproduction and somatic maintenance underlie life-history theory (Kirkwood and Austad, 2000; Kaplan and Robson, 2009).

Fast growth and high investment per reproductive event compromise individual lifespan (Metcalfe and Monaghan 2003; Austad 2010). At a within species-level studies on mice, rats and dogs selected for a fast growth or large body size found that they exhibit reduced longevity (Patronek et al 1997; Miller et al. 2000; Bartke et al. 2001a; Rollo 2002). Other experimental evolution studies have demonstrated negative associations between early-life fitness and longevity in *Drosophila* and other invertebrates (Rose and Charlesworth, 1980; Travers et al. 2015). At an among-species level, fast-growing species show short lifespans, compared with slow-growing ones (Bielby et al. 2007, Jones et al. 2008). Furthermore, a recent review that surveyed 26 studies, involving 24 different species of bird, mammal and

reptile, found 21 out of 26 studies detected a trade-off between early and late-life fitness (Lemaître et al. 2015), indicating that high investment in early reproduction has detrimental effects in late-life fitness. The fact that most studies of natural populations were able to detect the predicted trade-offs is surprising as selective mortality acting on poor condition individuals could remove them from the population, thus reducing the signal for low early-life reproductive effort, masking the existence of a trade-off. Greater understanding of the trade-offs that drive differences in longevity require disentangling the effects of growth and reproduction, ideally in a setting where the potential confounding effects of extrinsic mortality and individual differences in resource acquisition (van Noordwijk and de Jong 1986) are minimized.

The domestic dog represents a unique animal model as its biology has several distinctive aspects that are relevant for aging studies. Due to selective breeding there are almost 20-fold variation in body size and over two-fold differences in aging rates. Dogs and humans have co-evolved and share recent evolutionary selection processes, such as adaptation to digestion of starch rich diets (Axelsson et al. 2013), and there are clear signs of convergent evolution in the human and dog genome (Theofanopoulou et al., 2017). Purebred dogs can also be considered to be freed of extrinsic mortality, due to the absence of predators, general access to sufficient resources as well as often high-quality health care. Finally, unlike laboratory animals, dogs share the human environment and lifestyle, and are exposed to the same pollutants (Gilmore and Greer, 2015). Dogs present an extraordinary level of phenotypic variation in skeletal structure, including overall size, leg length, and variants of skull shape, even in comparison with all canids (Drake and Klingenberg,

2010). The largest dog breeds are more than one order of magnitude heavier than the smallest breeds, and litter sizes show five-fold variation while longevity differs more than two-fold among breeds (see Sup. Mat.). Previous studies have documented a decrease in lifespan with increasing body size across breeds (Speakman et al. 2003; Fleming et al. 2011; Greer et al. 2011; Selman et al. 2013), contrary to what is observed when comparing between mammalian species, but in accordance with reports comparing individuals from the same species from laboratory strains of mice (Miller et al., 2000) and some anecdotal comparisons among horses and humans (Masoro and Austad, 2010). The negative correlation between body size and lifespan has been attributed to fast growth (Kraus et al. 2013). However, it is unclear whether reproductive investment differences among dog breeds also influence lifespan when controlling for body size, reflecting the expected trade-off between reproduction and longevity.

Dog breeds present both an analytical challenge and an opportunity. The more than 400 described dog breeds can be considered as closed populations, with many breeds having been developed mainly during the past two hundred years as a result of artificial selection, reproductive separation, migration and hybridization (Parker et al. 2004, Spady and Ostrander 2008). Gene flow has been suggested to play a particularly important role in the immense phenotypic variation observed among breeds, occurring during natural hybridization between grey wolves and dogs, between so called local breeds from different geographic origin and with influence of different wolf populations, and within major clades of modern dogs (Vilà et al. 1997, Parker et al. 2004, Franz et al. 2016, Bergström et al. 2020). The aforementioned

gene flow means that the relationships between dog breeds are unlikely to be tree-like, thus there is no appropriate phylogeny to represent their relationships, and typical comparative approaches to control for non-independence of observations are unsuitable (Stone et al. 2011). Genomic analyses have allowed to disentangle the signatures of ancient shared ancestry and that of recent hybridization events, revealing the evolutionary history of dog breeds (Lindblad-Toh et al. 2005, Parker et al 2017). This novel genomic information also allows detailed analyses accounting for the potential confounding effects of both shared ancestry and gene flow, both of which violate the assumption of independence of observations (and residuals in linear models) (Felsenstein 2002; Stone et al. 2011). In addition, recent genomic information allows analyses of the contribution of shared ancestry and recent gene flow in shaping key phenotypic traits of dog breeds (e.g. Garamszegi et al. 2020).

Here we aim to analyze whether reproductive investment affects lifespan in dog breeds, as predicted by the disposable soma theory of aging. We hypothesize that breeds with high reproductive investment (i.e. litter size multiplied by neonate weight) present reduced lifespan, when controlling for adult weight. We employ a mixed model approach, following Garamszegi et al. (2020), which allows us to simultaneously account for non-independence due to shared ancestry and recent gene flow involved in the creation of new breeds (Felsenstein 2002, Stone et al. 2011, Larson et al. 2012, Parker et al. 2017). Using this modelling approach we were also able to explore the influence of shared ancestry, which mostly represents the ancient origin of main breed types, versus recent hybridization events, occurring

mostly during the last centuries, on key life-history traits among dog breeds (Parker et al. 2017; Garamszegi et al. 2020).

Methods

We collected data on lifespan (years), mean adult weight (kg, as an estimate of body size), mean neonate weight (kg), and mean litter size for as many dog breeds as possible (428 final rows, including varieties of the same breed and not recognized breeds) from the published literature. Given the different sources from which we obtained data, and the risk that criteria for data collection differed among sources, we first carefully checked the degree to which data from different sources were comparable based on the degree to which information from different sources for the same trait was correlated. Note we obtained different sample sizes when collecting data for each variable. The final sample size depended on the number of breeds for which we obtained data for all variables.

Lifespan

We collected lifespan information from the American Kennel Club official website (AKC, https://www.akc.org), and values from other published sources (Michell 1999, Bell et al. 2012, O´Neill et al. 2013, Leroy et al. 2015, Supplementary Material, Table S1). We tested the correlation among data sources for breeds for which we had information from more than one source, and discarded sources when the correlation with other sources was $r < 0.6$. We then averaged values from sources that met the minimum correlation criterion. Following this approach we were able to collect information on longevity for 277 dog breeds.

Adult weight

We collected mean adult weight, combining male and female weights (or using the mean breed-specific weight) as lifespan data was not sex-specific, from the AKC official website, and several published sources (Supplementary Material, Table S2). Since only recognized breeds are included (based on the criteria of the AKC), adult weight is expected to present limited variation, because it is highly related to pedigree parameters on which there is strong artificial selection, such as height. Although we acknowledge there is sexual size dimorphism in many breeds (more notably so in larger breeds), such dimorphism is small compared to among-breed differences in size, so it is highly unlikely to affect our results. Given the high correlation among adult weights from different sources ($r > 0.94$; see Supplementary Material, Table S2), we chose to use weights from the official AKC database. We collected information on adult weight for 253 dog breeds.

Neonate weight and litter size

Due to the scarcity of information from different published sources for the same breed for mean neonate weight and litter size, it was not possible to test the degree of pairwise correlation in these variables among different sources. Nonetheless, as an approximation we compared data collected from primary publications with that obtained from compendia, in order to verify whether values are comparable. For neonate weight the correlation between articles and compendia was $r = 0.84$, while the correlation for litter size was $r = 0.83$. We thus combined information from articles and compendia to maximize the sample size and calculated an average when we had more than one value for the same breed. Data for neonate finally came from

seven different sources and covered 281 breeds (Supplementary Material, Table S3). Data on litter size was obtained from 20 different sources (Supplementary Material, Table S4). In addition for 13 breeds (Picardy Sheepdog, Curly Coated Retriever, Coton de Tulear, Field Spaniel, Great Pyrenees, Irish Water Spaniel, Komondor, Kuvasz, Miniature Bull Terrier, Otterhound, Pharaoh Hound, Chinese Shar-Pei, and Schipperke) the litter sizes were obtained from two specialized websites (Supplementary Material, Table S4), as they were not available in other published sources. Litter sizes from the aforementioned web site were within the expected range for both breeds and adult weights, and both websites reported similar values, which gives us additional confidence that these are unlikely to be biased. We collected information on litter sizes for 253 breeds. Finally, we calculated total reproductive investment as the product of breed-specific litter size and neonate weights.

Growth rate

Previous work suggests that growth may compromise longevity in dogs (Galis et al. 2007; Kraus et al. 2013; Fan et al. 2016). Therefore, as a proxy for growth rate, we subtracted the adult weight from the neonate weight and divided the result by the neonate weight, to estimate how much a newborn individual must grow to reach average adult weight for each breed. Following this approach we collected information on growth rates for 124 breeds. A previous study (Hawthorne et al. 2004) followed individuals from 12 breeds during their lifetime, weighing them at different ages, and estimated growth curves by plotting mean body weight against age, fitting a logistic equation (see Hawthorne et al. 2004). This approximation was not possible

since such information does not exist for most breeds and would not be strictly comparable among different studies. Nonetheless, our estimate of growth is highly correlated with the estimated time to reach 99% of adult size (weeks) obtained from the logistic growth-curves of Hawthorne et al. (2004): $r = 0.89$, $n = 12$ breeds.

Shared ancenstry and recent gene flow

To estimate the influence of shared ancestry versus recent gene flow on differences in life-history traits among breeds, as well as to control for statistical non-independence of observations due to the aforementioned factors (Felsenstein 2002, Stone et al. 2011), we used novel genomic information (Parker et al. 2017) following Garamszegi et al. (2020). We used information from 150,067 informative SNPs, from which the origin of major clades of dog breeds can be reliably resolved (Parker et al. 2017). Degree of shared ancestry was based on distance data based on the proportion of allele comparisons that are not identical-by-descent. Garamszegi et al. (2020) have shown that such estimates are repeatable when multiple estimates for each pairwise between-breed comparison are obtained based on different pairs of individuals. We created a matrix where the off-diagonals represent median distances between breeds and the diagonal, representing comparisons within breeds, was filled with 0. Since analyses require a matrix describing expected covariances among breeds, we subtracted each value from 1 to obtain an expected similarity value (see Garamszegi et al. 2020 for further details).

As an estimate of the influence of the homogenizing effect of gene flow on life-history traits we used identical-by-descent haplotype sharing as estimated by 100-SNP windows (Parker et al. 2017). Haplotype sharing between breeds provides reliable

information on recent genetic admixture, as the length of haplotype sharing between breeds predicts history of between-breed crosses, because recombination events following admixture will slowly decay the length of such shared haplotypes. As for the SNP data above, Garamszegi et al. (2020) have shown that shared haplotype data reliably estimates between-breed similarities as they are significantly repeatable when estimated based on information from different individuals of the same breed (see Garamszegi et al. 2020). We created a matrix of haplotype sharing filling the off-diagonals with median of the pairwise between-breed haplotype sharing and the diagonals with the medians of within-breed haplotype sharing estimates from Parker et al. (2017). To scale the matrix so that estimates of haplotype sharing varied between 0 and 1, we recalculated each off-diagonal cell relative to the respective within-breed haplotype sharing values (see Garamszegi et al. 2020 for details). Both matrices were included in Bayesian mixed models as random factors (see below).

We incorporated the genomic data from Parker et al. (2017) to our previous database, obtaining data on lifespan, adult weight, neonate weight, litter size, reproductive investment, growth rates, and genomic data (SNPs and haplotype sharing matrices) for 92 different breeds with no missing data.

Statistical analyses

We used a Bayesian mixed modelling approach (Hadfield and Nakagawa 2010, Garamszegi et al. 2020) which allowed us to partition among-breed variation in life-history traits into variance components of evolutionary importance and control for non-independence of observations. We ran univariate models, with the aim of estimating the influence of shared ancestry and gene flow on key life-history traits

(see Garamszegi et al. 2020). We ran models where the life-history trait of interest was the response variable, without any fixed effects, and our estimates of shared ancestry and gene flow included as random factors, enabling us to partition variation around the estimated mean value of the life-history trait (intercept) into effects of shared ancestry and gene flow. Because we did not have repeated samples from a sufficient number of individuals within each breed, we did not include estimates of within-breed variation. We also used Bayesian mixed models, including shared ancestry and gene flow as random factors, for all analyses of the relationship between life-history traits and longevity. For these models we first standardized all variables for ease of interpretation, as some models included interaction terms (Schielzeth 2010). We fitted all models using the MCMCglmm package (Hadfield 2010) in R (R Core development team 2020). Prior to entering the matrices of expected similarity due to shared ancestry or gene flow, we applied single value decomposition on each matrix (see Garamszegi et al. 2020). We defined weakly informative priors for all models: G: V = 1, nu = 1, R: V = 1, nu = 0.002. We ran models for 400,000 iterations, with a thining interval of 50, and discarded the first 70,000 iterations as burnin. The trace and distribution of all parameters was checked visually. For all analyses we had effective sample size (i.e. the number of effectively independent draws from the posterior distribution) ranging between 5600 – 7281.

Results

Influence of shared ancestry and hybridization on life-history traits.

The univariate mixed models indicated that most of the variation in key life-history traits among dog breeds is the result of common ancestry (estimated by shared

SNPs), which explained 74.7% – 97.2% of the among-breed variation. In contrast, recent gene flow (estimated by haplotype sharing) explained 2.6% - 24.2% of the variation (see Table 1). Lifespan and adult weight showed the highest influence of shared ancestry (95.36 - 97.15%), while neonate weight the lowest (74.67%)

Table 1. Bayesian mixed models on lifespan, weight, litter size, and neonate weight, respectively. Estimates of shared ancestry (based on shared SNPs) and recent gene flow (based on haplotype sharing) were included as random effects. Shown are: the effective sample sizes (eff.samp) for all parameters, their posterior means (post.mean), the 95% credibility intervals (95% CrI), the proportion of the variance partitioned among the random effects and the residual variance (% var.explained), and for the fixed effect (intercept) an estimate of the p value (pMCMC).

Lifespan				
Random effects	**eff.samp**	**post. mean**	**95% CrI**	**% var.explained**
Shared ancestry	5645	17.41	11.14, 24.19	97.15
Gene flow	5380	0.47	0.08, 0.98	2.61
Residual variance	1440	0.04	0.00, 0.19	0.24
Fixed effects				**pMCMC**
Intercept	6600	9.32	2.53, 15.99	**0.008 ****
Weight				
Shared ancestry	6019	12.58	7.17, 18.79	95.36
Gene flow	5387	0.56	0.12, 1.11	4.27
Residual variance	1510	0.05	0.00, 0.19	0.36
Fixed effects				**pMCMC**
Intercept	6600	5.75	0.07, 11.43	**0.04 ***

Litter size				
Shared ancestry	1932	7.80	0.08, 18.24	79.69
Gene flow	2033	1.92	0.60, 3.25	19.68
Residual variance	1386	0.062	0.00, 0.28	0.64
Fixed effects				**pMCMC**
Intercept	6600	5.34	0.34, 10.32	**0.047 ***
Neonate weight				
Shared ancestry	6600	0.07	0.03, 0.10	74.67
Gene flow	6600	0.02	0.01, 0.02	24.15
Residual variance	5917	0.00	0.00, 0.00	1.18
Fixed effects				**pMCMC**
Intercept	6600	0.62	0.13, 1.03	**0.006****

Lifespan and weight

We confirmed the previously reported negative relationship between weight and lifespan across dog breeds, even when accounting for shared ancestry and recent gene flow (see Table S5, supplementary material). Our results indicate that most of the variance within the relationship between adult weight and longevity is explained by common ancestry (72.79%) compared with the influence of recent gene flow (25.4%).

Lifespan and reproductive investment

Lifespan decreases with higher reproductive investment (product of litter size and neonate weight; see Table 2). Note that we could not include adult weight in the analysis due to collinearity, given the high correlation between neonate weight and

adult weight (r = 0.87, without controlling for non-independence of observations), thus we cannot rule out that we are detecting an effect of weight on longevity, if larger breeds also invest more in reproduction. Most of the variance in the aforementioned model is explained by shared ancestry compared with recent gene flow.

Table 2. Bayesian mixed model of the relationship between lifespan (dependent variable) and reproductive investment (litter size x neonate weight: rep invest) among dog breeds. Reproductive investment is a fixed effect. Estimates of shared ancestry (based on shared SNPs) and recent gene flow (based on haplotype sharing) were included as random effects. Shown are: the effective sample sizes (eff.samp) for all parameters, their posterior means (post.mean), the 95% credibility intervals (95% CrI), the proportion of the variance partitioned among the random effects and the residual variance (% var.explained), and for the fixed effect an estimate of the p value (pMCMC).

Random effects	**eff.samp**	**post. mean**	**95% CrI**	**% var.explained**
Shared ancestry	3476	1.459	0.13, 3.22	77.42
Gene flow	5380	0.47	0.08, 0.98	21.25
Residual variance	1440	0.04	0.00, 0.19	1.33
Fixed effects				**pMCMC**
Intercept	4665	-0.74	-3, 1.44	0.499
Rep invest	4776	-0.536	-0.71, -0.35	**<2e-04 *****

Lifespan, adult weight, and litter size

To attempt to disentangle a size effect on longevity from the effect of reproductive investment on lifespan, we tested the relationship between litter size and lifespan, controlling for adult body weight, including an interaction between adult weight and litter size. We found a significant interaction between litter size and adult weight affecting lifespan, indicating that the reduction in lifespan resulting from larger litter sizes is dependent on adult weight (see Table 3), with larger breeds showing a higher reduction in lifespan with increased litter size compared to smaller breeds. Again most of the variance in lifespan is explained by shared ancestry compared with recent gene flow in this model.

Table 3. Bayesian mixed model of the relationship between adult weight (weight, square root transformed), litter size (LS) and lifespan among dog breeds. Adult weight and litter size are fixed effects and their interactive effect on lifespan was also tested. Estimates of shared ancestry (based on shared SNPs) and recent gene flow (based on haplotype sharing) were included as random effects. Shown are, the effective sample sizes (eff.samp) for all parameters, their posterior means (post.mean), the 95% credibility intervals (95% CrI), the proportion of the variance partitioned among the random effects and the residual variance (% var.explained), and for the fixed effect an estimate of the p value (pMCMC).

Random effects	**eff.samp**	**post. mean**	**95% CrI**	**% var.explained**
Shared ancestry	6051	0.81	0.11, 1.67	75.32
Gene flow	5654	0.24	0.10, 0.37	22.4
Residual variance	2330	0.02	0.00, 0.09	2.28
Fixed effects				**pMCMC**
Intercept	6600	-0.37	-2.04, 1.22	0.66
Weight	6600	-0.95	-1.16, -0.74	**<2e-04 *** **
LS	7154	0.23	0.03, 0.42	0.01*
weight:LS	7013	-0.24	-0.38, -0.10	**0.0003*** **

Lifespan, growth and reproductive investment

Because both growth and reproduction may influence lifespan (Stearns et al. 2000, Metcalfe and Monaghan, 2003), we carried out a final model to test the relationship between lifespan and growth and reproductive investment, and the interaction between growth and reproductive investment affecting lifespan. The interaction between growth and reproductive investment was statistically significant (Table 4), indicating that fast-growing breeds suffer greater reduction in lifespan with higher reproductive investment than slow-growing breeds (Table 4). Most of the variance in lifespan is explained by shared ancestry when compared to gene flow in this model.

Table 4. Bayesian mixed model of the relationship between growth and reproductive investment among dog breeds. Growth and reproductive investment (Rep invest) are fixed effects and their interaction was also tested. Estimates of shared ancestry (based on shared SNPs) and recent gene flow (based on haplotype sharing) were

included as random effects. Shown are, the effective sample sizes (eff.samp) for all parameters, their posterior means (post.mean), the 95% credibility intervals (95% CrI), the proportion of the variance partitioned among the random effects and the residual variance (% var.explained), and for the fixed effect an estimate of the p value (pMCMC).

Random effects	**eff.samp**	**post. mean**	**95% CrI**	**% var.explained**
Shared ancestry	5808	0.88	0.11, 1.67	75.07
Gene flow	4470	0.26	0.10, 0.37	22.07
Residual variance	2261	0.03	0.00, 0.09	2.85
Fixed effects				**pMCMC**
Intercept	5400	-0.50	-2.25, 1.17	0.56
Growth	7281	-0.43	-0.62, -0.24	**<2e-04 *** **
Rep invest	6777	-0.32	-0.52, -0.13	**0.001** **
Growth : Rep invest	6330	-0.32	-0.48, -0.16	**<2e-04 *** **

Discussion

We found that among-breed variation in key life-history traits is mainly explained by shared ancestry (range: 75 – 92 % of the variance), as measured by SNPs that are identical by descent, a measure of overall genetic similarity. On the other hand, recent gene flow, measured based on shared haplotypes, had a more reduced influence (range: 3 – 24%). Adult body weight and median lifespan showed the highest influence of shared ancestry, while litter size showed the lowest. Our results

further indicate that reproductive investment influences lifespan, but the effect is dependent on breed weight and growth rate. Larger, faster-growing breeds which invest more in reproduction pay a higher price in reduced lifespan than smaller, slow-growing breeds. To illustrate, a Saint Bernard has on average 9.4 pups per litter, with neonates that weigh ca. 0.7% of adult body weight (~65.7 kg), and live an average 6.4 years, while a Toy Poodle has ~2.2 pups per litter, but neonates already weigh ca. 5.1% of adult body weight (~1.5 kg), and live an average of 14.7 years.

Shared ancestry, reflecting the ancient evolutionary history of dog breeds through processes of artificial selection or neutral processes, plays a more important role in explaining the inter-breed variation in key life-history traits, compared with recent events of admixture. These results are in line with Garamszegi et al. (2020), who studied a behavioural trait and found that common ancestry has a considerable role on the among-breed variance in human-directed play behavior (~80%), when compared to relatively recent gene flow (~18%).

The apparent minor influence of admixture could seem surprising, as gene flow in purebred dogs reflects artificial selection directed by humans to obtain or refine specific, desired phenotypes, resulting in different breeds. Thus, it would be reasonable to expect some degree of homogenization of particular phenotypic traits as a result of such admixture among breeds. It is possible that crosses among different breeds involved differences in specific phenotypic traits breeders wanted to incorporate into the newly created breeds, but involved more minor differences in life history traits, such as neonate weight for example, as more pronounced differences in such traits could lead to problems. Finally, it is also possible that inter-breed

crosses that occurred more distantly in the past become harder to identify, and thus we only observe the effects of more recent crosses (Parker et al. 2017), diluting potential effects on life history traits. For example, breeds with a more recent history share a larger proportion of haplotypes, such as the Bullmastiff with the Mastiff and Bulldog, or the Golden Retriever with both the Flat-Coated Retriever and Irish Water Spaniel, or the Chinook with the German Sheperd and Greenland Sledgedog (Parker et al. 2017).

The difference in the relative magnitude of the influence of recent gene flow on the amount of among-breed variation in lifespan and adult weight (2.6 and 4.3%, respectively) in comparison with litter size and neonate weight (19.7 and 24.1%, respectively) is interesting. This difference suggests that admixture had a stronger influence on traits related with reproduction compared with adult body weight or lifespan. It is possible that lifespan is more strongly correlated with adult weight than litter size or neonate weight, and thus reflects more closely the artificial selection imposed on adult weight. It is also possible that directed hybridization in the formation of modern breeds involved crosses between breeds of similar size to avoid "mechanical" problems associated to large size differences, whereas other traits had a much larger differences. Besides artificial selection directed by humans, a comparison of the effects of shared ancestry and recent gene flow could be useful in future studies to trace the evolution of specific traits which are correlated or interact with each other, as predicted by the domestication syndrome (Wilkins et al. 2014; Theofanopoulou et al. 2017).

We also found a negative association between reproduction and lifespan among dog breeds. We first confirmed the previously reported negative association between lifespan and adult body size (Speakman et al. 2003; Fleming et al. 2011; Greer et al. 2011; Selman et al. 2013), even when controlling for the confounding effects of shared ancestry and gene flow. We then tested the association between lifespan and reproductive investment, finding a negative association between these traits, where breeds with large litters of small neonates presented shorter lifespans than breeds with small litters of large neonates. To rule out that these results merely captured the effects of adult weight, which we could not include in the model due to collinearity, rather than reproduction *per se*, we also analyzed the relationship between lifespan and litter size, including adult body weight, as well as the interaction between litter size and adult weight affecting lifespan. Our results indicate that larger breeds that invest in larger litters, such as Saint Bernard or Great Dane, show a steeper decrease in lifespan compared with small breeds that invest in larger litters, such as the Griffon Bruxellois or miniature Pinscher. These results support the idea of a trade-off between reproduction and lifespan, as predicted by the disposable soma theory of aging (Kirkwood, 2017), and that such a trade-off is affected by the weight of the dog breed, with larger breeds paying a higher lifespan costs.

Previous work suggested that the negative association between adult weight and longevity is a result of the increased growth rate that large breeds require to reach adult weight, which might have led to shorter lifespan due to specific developmental diseases or faster aging processes (Galis et al. 2007, Kraus et al. 2013). The lower

ratio between neonate weights relative to adult weight of large breeds, in comparison with smaller ones, means that more energy needs to be invested in growth to reach adult size, which leads to lower investment in somatic maintenance, thus reducing lifespan (Fan et al. 2016). Based on these previous results we also included a final model to test whether the observed relationship between reproductive investment and lifespan holds when including a proxy for growth rate (the ratio between neonate and adult body weight), instead of adult weight. We again found that higher reproductive investment is associated with reduced lifespan, but the effect depended on our proxy for growth rate, where fast-growing breeds, such as the Saint Bernard or Rottweiler, showed a steeper decline in lifespan with greater reproductive investment compared with slow-growing breeds, such as the Coton de Tulear or Papillon. It is worth noting that our results indicating a reduction in lifespan associated with reproduction are in line with evidence that sterilization increases lifespan by 13.8 % in male dogs and by 26.3% in females (Hoffman et al. 2013).

According to the disposable soma theory, aging is the result of the trade-off in the allocation of limited resources between two competing functions: reproduction and somatic maintenance. Organisms are not immortal because investing into error-proof somatic maintenance is wasteful and not an evolutionarily stable strategy, as extrinsic mortality can terminate even intrinsically immortal organisms (Maklakov and Immler 2016). Physiological deterioration is accelerated by fast growth and high investment into reproduction, resulting in reduced lifespan. There is ample evidence for the detrimental effects of fast growth and development on lifespan. For example, artificial selection for fast growth in fruit flies (*Drosophila melanogaster*) and mice

(*Mus musculus*) results in decreased lifespan (Stearns et al. 2000, Miller 2000), and increased embryo growth rates are associated with shorter lifespans in birds and mammals (Ricklefs, 2006). Differences in lifespan across dog breeds were also found to be associated with differences in growth rate (Kraus et al. 2013). On the other hand, the costs of reproduction involve not only investment into gamete production but also the 'wear-and-tear' of tissues, DNA damage from free radicals and accumulation of toxic waste products in the cells (reviewed in Maklakov and Immler 2016). Our results suggest that high investment into reproduction can explain among-breed differences in lifespan, as higher reproductive investment is associated with reduced lifespan, with larger breeds showing a steeper trade-off between reproductive investment and lifespan compared with smaller breeds, even when controlling for shared ancestry and gene flow. Why do we observe an interaction with adult weight, rather than merely additive effects? It is possible that greater reproductive investment imposes a steeper trade-off with lifespan in larger breeds because individuals from such breeds have already traded-off self-maintenance with growth early in their life-time, or because the cost of reproduction is compounded with developmental diseases that result from fast growth rate (Kraus et al. 2013; Fleming et al. 2011; Farrell et al. 2015). It is interesting that small dog breeds do not seem to trade-off lifespan with investment into reproduction, in fact our model suggest small breeds with larger litters might actually live longer than small breeds with small litters. It is possible that other variables are influencing litter sizes on small breeds, such as the specific shape of the skull and / or the proportional size of the head in comparison to body size. Both brachycephalic and dolicocephalic skulls have been associated with an increased risk of dystocia (i.e. difficulty giving

birth) and the need of caesarean section. Risk of dystocia was found to be the highest in the Scottish Terrier, Chihuahua, Pomeranian, Pug and Staffordshire Bull Terrier (Bergström et al. 2006), none of which are large breeds. Such reproductive problems might be constraining reproductive investment independently of lifespan.

We cannot rule out that the observed association between lifespan and reproductive investment is merely a correlated response of the effects of body weight on lifespan, given the strong association between body weight and both lifespan and reproductive investment. However, the interaction between adult weight and litter size, as well as the interaction between growth rate and reproductive investment, in the relationship with lifespan, suggest that the association between reproduction and lifespan is not merely a correlated response to body size.

Conclusion

In sum, our results indicate that shared ancestry, as estimated as SNPs that are identical by descent, explains a higher proportion of the among-breed variation in key life-history traits, in comparison with recent gene flow, estimated as haplotype sharing. These results suggest that recent crosses between pre-existing breeds (Parker et al. 2017) have left a minor imprint on life-history traits. Interestingly, litter size and neonate weight, showed a much higher influence (> 4x higher) of hybridization compared with adult body weight and lifespan. Our results also show that investment in reproduction negatively impacts lifespan, and more strongly so in large breeds. The interaction between adult weight, or growth, and reproduction on lifespan suggests the effect is not merely a correlated response of the effect of adult weight on lifespan. These results are in line with predictions from the disposable

soma theory for the evolution of aging and suggest that among-breed differences in lifespan are due to a combination of body weight and investment into reproduction. The precise mechanisms involved require further investigation.

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Supplementary Material

Table S1. Lifespan sources.

Lifespan		
Source	**breeds (n =)**	**correlation**
https://www.akc.org	270	r > 0.6
Michell,1999	66	
Bell et al. 2012	176	
O´Neill et al. 2013	35	
Leroy et al. 2015	7	
Total (different breeds)	277	

Table S2. Adult weight sources.

Adult Weight		
Source	**breeds (n =)**	**correlation**
https://www.akc.org	253	r > 0.94
Speakman et al. 2003	3	
Hawthorne et al. 2004	12	
Gerstner et al. 2010	31	
Bell et al. 2012	177	
Kraus et al. 2013	74	
Fan et al. 2016	89	
Total (different breeds)	281	

Table S3. Neonate weight sources

Neonate Weight		
Source	**breeds (n =)**	**correlation**
Compendia	39	r = 0.84
Clark et al. 2017a		
Clark et al. 2017b		
Clark et al. 2017c		
Scientific Articles	188	
Yilmaz, 2007		
Groppetti et al. 2015		
Fan et al. 2016		
Groppetti et al. 2017		
Total (different breeds)	152	

Table S4. Litter size sources.

Litter size			
Source	**breeds (n =)**		**correlation**
Compendia		39	r = 0.83
Bell et al. 2012	1		
Clark et al. 2017a	38		
Clark et al. 2017b			
Clark et al. 2017c			
Scientific Articles		383	
Wildt et al. 1982	1		
Okkens et al. 1993	5		
Nielen et al. 2001	5		
Thomassen et al. 2006	6		
Yilmaz, 2007	1		
Ograk, 2009	1		
Gubbels et al. 2009	12		
Borge et al. 2011	199		
Groppetti et al. 2015	30		
Leroy et al. 2015	7		
Mila et al. 2015	14		
Goleman et al. 2015	8		
Schrack et al. 2017	1		
Groppetti et al. 2017	93		
Websites		13	
https://www.dogbreedinfo.com	13		
http://www.easypetmd.com	13		
Total (different breeds)	253		

Table S5. Bayesian mixed model of the relationship between lifespan (dependent variable) and adult weight among dog breeds. Adult weight is a fixed effect. Estimates of shared ancestry (based on shared SNPs) and recent gene flow (based on haplotype sharing) were included as random effects. Shown are: the effective sample sizes (eff.samp) for all parameters, their posterior means (post.mean), the 95% credibility intervals (95% CrI), the proportion of the variance partitioned among

the random effects and the residual variance (% variance explained), and for the fixed effect an estimate of the p value (pMCMC).

Random effects	**eff.samp**	**post. mean**	**95% CrI**	**% var.explained**
Shared ancestry	4551	0.882	0.11, 1.9	73.13
Gene flow	4522	0.302	0.14, 0.45	25.04
Residual variance	1784	0.022	0.0002, 0.09	1.82
Fixed effects				**pMCMC**
Intercept	5000	-0.54	-2.27, 1.14	0.52
Rep invest	5240	-0.71	-0.87, -0.57	**<2e-04 *****

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Discusión

Confirmamos la asociación negativa reportada anteriormente entre longevidad y peso del adulto (Speakman et al. 2003; Fleming et al. 2011; Greer et al. 2011; Selman et al. 2013), incluso controlando por los efectos de la ascendencia compartida y el flujo génico que caracterizan la historia evolutiva de los perros. Luego exploramos la relación entre la longevidad y la inversión reproductiva, encontrando una asociación negativa entre estos rasgos ($p < 2e\text{-}04$). Sin embargo, debido a problemas de multicolinealidad, no pudimos controlar las diferencias con el peso del adulto y, por lo tanto, no podemos descartar que estos resultados simplemente reflejan los efectos del tamaño del adulto sobre la longevidad, en lugar de la reproducción *per se*. Por lo tanto, también analizamos la relación entre la longevidad y el tamaño de la camada, controlando por el peso del adulto, así como el efecto de la interacción entre el tamaño de la camada y el peso del adulto. Nuestros resultados indican que las razas más grandes que invierten en camadas más grandes muestran una disminución más pronunciada de la longevidad, en comparación con las razas pequeñas que invierten en camadas más grandes ($p < 0.005$). Estos resultados apoyan la idea de una disyuntiva entre reproducción y longevidad, tal como predice la teoría del envejecimiento del soma desechable (Kirkwood, 2017), pues el efecto de la inversión reproductiva sobre la longevidad depende del peso del adulto, siendo las razas más grandes aquellas con costos más elevados.

Trabajos anteriores han sugerido que la asociación negativa entre el tamaño del adulto y la longevidad es el resultado de una mayor tasa de crecimiento por parte de las razas grandes para alcanzar el tamaño adulto, lo que conduce a una menor longevidad debido a enfermedades específicas del desarrollo o procesos de envejecimiento más rápidos (Galis et al. 2007, Kraus et al.2013). Además, una mayor diferencia entre el peso del neonato y el peso del adulto por parte de las razas grandes, en comparación con las razas pequeñas, sugiere que se necesita invertir más energía en el crecimiento para alcanzar el tamaño relativo del adulto, lo que conduce a menor inversión en el mantenimiento somático, reduciendo así la

longevidad (Fan et al. 2016). Con base en estos resultados previos, incluimos un modelo para probar si la relación observada entre la inversión reproductiva y la longevidad se mantiene cuando incluimos una estima de la tasa de crecimiento (la diferencia entre el peso del adulto y el peso del neonato, dividida entre el peso del adulto), en lugar de solamente el peso del adulto. Nuevamente encontramos que la inversión reproductiva está asociada con una menor longevidad, pero el efecto depende de la tasa de crecimiento, donde las razas con mayor crecimiento mostraron una disminución más pronunciada de la longevidad a mayor inversión reproductiva, en comparación con las razas de menor crecimiento. Vale la pena señalar que nuestros resultados que indican una reducción en la longevidad asociada con la reproducción complementan la evidencia de que la esterilización aumenta la longevidad en un 13.8% en perros machos y en un 26.3% en hembras (Hoffman et al. 2013).

De acuerdo con la teoría del soma desechable, el envejecimiento es el resultado de la compensación en la asignación de recursos limitados entre dos funciones que compiten entre sí: reproducción y mantenimiento somático. Los organismos no son inmortales porque invertir en un mantenimiento somático a prueba de errores es un sinsentido, pues la mortalidad extrínseca continuará eliminando organismos aunque éstos sean intrínsecamente inmortales (Maklakov e Immler 2016). El deterioro fisiológico se acelera mediante un crecimiento rápido y una alta inversión en la reproducción, lo que reduce la longevidad del individuo pero introduce soluciones evolutivamente estables para enfrentar la mortalidad extrínseca a nivel poblacional. Existe amplia evidencia de los efectos perjudiciales del crecimiento rápido sobre la longevidad. Por ejemplo, la selección artificial hacia un crecimiento rápido en moscas (*Drosophila melanogaster*) y ratones (*Mus musculus*) da como resultado una menor longevidad (Stearns et al. 2000, Miller 2000), y el aumento de las tasas de crecimiento embrionario se asocia con una menor esperanza de vida en aves y mamíferos (Ricklefs 2006). Asimismo existe evidencia de que las diferencias en la longevidad entre razas de perros están asociadas con diferencias en la tasa de crecimiento (Kraus et al. 2013). Por otro lado, los costos de reproducción implican no solo la inversión en la producción de gametos, sino también el 'desgaste' de los

tejidos, el daño al ADN por parte de radicales libres y la acumulación de diversos desecho tóxicos de las células (revisado en Maklakov e Immler 2016). Nuestros resultados sugieren que una alta inversión en la reproducción también explica ciertas diferencias entre razas sobre la longevidad, dado que una mayor inversión reproductiva se asocia con una longevidad reducida, y las razas más grandes muestran una compensación más pronunciada entre la inversión reproductiva y la longevidad en comparación con las razas más pequeñas, incluso cuando se controla por la ascendencia compartida y el flujo génico. ¿Por qué observamos un efecto de interacción con el peso del adulto, en lugar de efectos meramente aditivos? Es posible que una mayor inversión reproductiva imponga una compensación más pronunciada sobre la longevidad en razas más grandes, porque los individuos de tales razas ya han intercambiado el mantenimiento somático por un mayor crecimiento en su vida, o porque el costo de reproducción se agrava con el desarrollo de enfermedades que son más comunes con mayores tasas de crecimiento (Kraus et al. 2013; Fleming et al. 2011; Farrell et al. 2015).

No podemos descartar que la asociación observada entre la longevidad y la inversión reproductiva sea simplemente una respuesta correlacionada de los efectos del tamaño corporal sobre la longevidad, dada la fuerte asociación entre el peso del adulto tanto con la longevidad como con la reproducción. Sin embargo, la interacción entre el peso del adulto y el tamaño de la camada, así como la interacción entre la tasa de crecimiento y el peso del adulto, en relación con la longevidad, sugieren que la asociación entre la reproducción y la longevidad no es simplemente una respuesta correlacionada, pues el efecto de la reproducción difiere dependiendo del tamaño de las razas de perro.

En resumen, nuestros resultados muestran que la reproducción impacta negativamente sobre la longevidad dependiendo del tamaño del perro, afectando más fuertemente en las razas grandes. La interacción entre el peso y/o crecimiento del adulto y la inversión reproductiva sobre la longevidad, sugiere que dicho efecto no es simplemente una respuesta correlacionada del efecto del tamaño corporal sobre la longevidad. Estos resultados concuerdan con las predicciones de la teoría

del soma desechable sobre la evolución del envejecimiento, y sugieren que las diferencias entre razas en la longevidad se deben a una combinación entre tamaño del adulto e inversión reproductiva.