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SYNTHESIS PAPER

Junk DNA Contribution to Evolutionary Capacitance Can Drive Species Dynamics

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Abstract Junk DNA is still an enigmatic concept. Although junk DNA composition, abundance, and functionality are still contentious, its contribution to biological evolution is less questionable. Recently, I proposed that sexually restricted chromosomes such as *Y* and *W*, highly enriched in junk DNA elements, act as genomic tuning knobs indirectly causing a genome-wide increase in gene expression heterogeneity that boosts heterogametic individuals ability to endure environmental challenges and evolutionary capacitance, i.e., the store of genetic variation with no phenotypic effect. Sexually restricted chromosomes-based evolutionary capacitance might importantly contribute to metazoan sexual dimorphisms for dispersal and sex-biased gene expression dynamics. In this Synthesis, I hypothesize that large differences between species in the overall amount of junk DNA within their genomes also promote differences in junk DNA-based evolutionary capacitance that might be reflected in differences for dispersal and genetic diversification. I hypothesize that populations for species with junk DNA-impoverished genomes would show an enhanced ability to genetically diversify leading to a faster speciation rate even in the absence of geographic isolation when compared with populations for species with junk DNA-enriched genomes. To support junk DNA variation-based evolutionary capacitance effect on species genetic diversification, I analyzed the covariation of genome size as proxy for the overall amount of junk DNA in the genome and several genetic diversification measures obtained from interspecific crosses for the Drosophilidae family. The potential effect of junk DNA variation-based evolutionary capacitance for other elements of species dynamics such as extinction or the participation in grouped ecological structures is also briefly discussed.

Keywords Junk DNA · Genomic tuning knobs · Evolutionary capacitance · Heterochromatin · Gene expression heterogeneity · Speciation · Dispersal · Extinction

Introduction

A long-standing question in biology is how phenotypic traits relate with genotypic variation. The existence of genetic variation with no phenotypic effect or cryptic genetic variation is particularly challenging for the study of the connection between genotype and phenotype, and, consequently, the understanding of the etiology and expression of diseases, and the spatiotemporal dynamics of natural populations, or the development of programs for synthetic biology, or crop and livestock improvement (Gibson and Reed 2008; Masel and Trotter 2010; Paaby and Rockman 2014). Evolutionary capacitance is commonly defined as the accumulation of cryptic genetic variation, and its revelation under permissive circumstances (Gibson and Reed 2008; Masel and Trotter 2010; Paaby and Rockman 2014). Factors mediating evolutionary capacitance or evolutionary capacitors can be assorted in two classes depending on their mode of action. On one hand, phenotypic robustness-promoting capacitors can modulate cryptic genetic variation by making genetic variants phenotypically silent

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(Gibson and Reed 2008; Masel and Trotter 2010; Paaby and Rockman 2014). For example, chaperones guiding protein folding can ensure that proteins encoded by different variants of the same gene are folded in iso-functional structures (Gibson and Reed 2008; Masel and Trotter 2010; Paaby and Rockman 2014). More liberal folding caused by stressful situations that overwhelm chaperone action would permit protein variants becoming phenotypically distinguishable (Gibson and Reed 2008; Masel and Trotter 2010; Paaby and Rockman 2014). On the other hand, phenotypic heterogeneity-promoting capacitors can modulate cryptic genetic variation by causing phenotypic variation in natural populations in the absence of genetic variation (Ehrenreich and Pfennig 2016; Feinberg and Irizarry 2010; Forsman 2015; Kaern et al. 2005; Kelly et al. 2012; Kilfoil et al. 2009; Nonaka et al. 2015; Raj and van Oudenaarden 2008; Raser and O'Shea 2005; Schlichting and Wund 2014). For example, stochastic transitions at the molecular level can result in phenotypic variation even for populations of genetically identical individuals maintained in the same environment (Feinberg and Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009; Raj and van Oudenaarden 2008; Raser and O'Shea 2005). The spectrum of non-genetic phenotypes in natural populations allows them better coping with variable environments while permitting random drift of genetic variants that produce indistinguishable phenotypes (Ehrenreich and Pfennig 2016; Feinberg and Irizarry 2010; Forsman 2015; Kaern et al. 2005; Kelly et al. 2012; Kilfoil et al. 2009; Nonaka et al. 2015; Raj and van Oudenaarden 2008; Raser and O'Shea 2005; Schlichting and Wund 2014). Any factor narrowing the non-genetic phenotypic spectrum can make cryptic genetic variants phenotypically distinguishable, and, therefore, detectable for selective forces (Ehrenreich and Pfennig 2016; Feinberg and Irizarry 2010; Forsman 2015; Kaern et al. 2005; Kelly et al. 2012; Kilfoil et al. 2009; Nonaka et al. 2015; Raj and van Oudenaarden 2008; Raser and O'Shea 2005; Schlichting and Wund 2014). Recently, I suggested that metazoan species might show a generalized sexual dimorphism in evolutionary capacitance ultimately dependent on junk DNA deposits specific of heterogametic individuals promoting phenotypic heterogeneity (Diaz-Castillo 2015).

Since its inception, the junk DNA concept has been surrounded by a controversy that in essence is rooted on its use to refer to a potpourri of very diverse genetic elements with little in common other than questionable functionalities for the benefit of the individual, and the disparate definitions biological disciplines have for "function" (Doolittle 2013; Doolittle et al. 2014; Graur et al. 2013, 2015; Kellis et al. 2014; Niu and Jiang 2013; Palazzo and Gregory 2014). Junk DNA elements can be non transcribable like pseudogenes or highly repetitive DNA, transcribable but not translatable like introns, or even contain transcribable and translatable units devoted to their own selfish multiplication like transposable elements (Doolittle 2013; Doolittle et al. 2014; Graur et al. 2013, 2015; Kellis et al. 2014; Niu and Jiang 2013; Palazzo and Gregory 2014). As diverse as junk DNA elements can be, so are their effects as evolutionary facilitators (Biemont 2010; Doolittle and Sapienza 1980; Jurka et al. 2007; McClintock 1984; Ohno 1972; Orgel and Crick 1980; Yun et al. 2006; Oliver and Greene 2009; Bohne et al. 2008). Junk DNA elements can directly relate with genetic changes by being exapted into new exons, genes, or regulatory elements, or by participating in chromosome rearrangements that alter gene copy number and/or linear arrangement (Biemont 2010; Doolittle and Sapienza 1980; Jurka et al. 2007; McClintock 1984; Ohno 1972; Orgel and Crick 1980; Yun et al. 2006; Oliver and Greene 2009; Bohne et al. 2008). Junk DNA elements can also act as indirect modulators for the activity of other genetic elements. In 1997, David King and coworkers proposed the genetic tuning knob concept to refer to the modulator effect that the inherent, frequent and reversible variation in simple sequence repeats (SSRs) copy number might have on the functionality of genetic elements that are located close to or contain SSRs (Gemayel et al. 2010; Kashi and King 2006; King et al. 1997). Building on the tuning knob concept, I proposed that chromosomes found in only one sex like *Y* or *W, Y/W* chromosomes hereinafter, act as tuning knobs at a genomic scale because their frequent variation in junk DNA content indirectly promotes gene expression heterogeneity in other genes across the genome without further changes in these genes coding or direct regulatory sequences (Diaz-Castillo 2015).

Despite *Y/W* chromosomes originated from autosomes multiple times, they all proceed through a progressive inactivation characterized by the loss of coding genes, the enrichment in junk DNA elements such as highly repetitive DNA or transposable elements, and the increase in highly compacted chromatin or heterochromatin (Bachtrog 2013; Ellegren 2011; O'Meally et al. 2010; Papadopulos et al. 2015; Steinemann and Steinemann 2005; Mank 2012). The amount of junk DNA in *Y/W* chromosomes is considerably variable even for individuals of the same natural population or lab strain (Cohen et al. 2005; Cohen and Segal 2009; Halfer 1981; Hughes and Rozen 2012; Lyckegaard and Clark 1989, 1991; Paredes et al. 2011; Repping et al. 2006; Sahara et al. 2012; Singh et al. 1980; Nova et al. 2002). Such accentuated variation might depend on the very active production of DNA breaks and the preferential use of error-prone repair strategies in germ line nuclei where *Y/W* chromosomes are more accessible (Cohen and Segal 2009; Diaz-Castillo 2013; Diaz-Castillo and Ranz 2012; Lieber 2010; Peng and Karpen 2007; Preston et al. 2006; Suzuki et al. 2009).

Large repositories of heterochromatin such as *Y*/*W* chromosomes have been suggested to act as heterochromatin-forming elements sinks because they detract an important fraction of these elements from the nuclear pool (Berloco et al. 2014; Francisco and Lemos 2014; Marcand et al. 1996; Schaafsma and Pfaff 2014; Wijchers et al. 2010; Zuckerkandl 1974). *Y*/*W* chromosomes sink effect for heterochromatin-forming elements might be particularly influential during the first zygotic divisions because heterochromatin formation then depends on limited maternally-deposited material (Banaszynski et al. 2010; Baroux et al. 2008; Tadros and Lipshitz 2009), and heterochromatin-forming elements assortment that early can be maintained in later stages of development (Golic et al. 1998; Maggert and Golic 2002).

Because *Y*/*W* chromosomes junk DNA content might be considerably variable between gametes, the amount of heterochromatin-forming elements detracted from the limiting maternally-deposited pool towards *Y*/*W* chromosomes, and therefore the amount of this material left to be deployed in other loci out of *Y*/*W* chromosomes would also be variable between zygotes (Diaz-Castillo 2015). The zygotic variation in the amount of heterochromatin-forming elements that is deployed in non *Y*/*W* loci would be translated in a variation between individuals in chromatin compaction, and, therefore, transcription machinery accessibility for these loci from early embryogenesis onwards (Diaz-Castillo 2015). Thus, *Y*/*W* chromosomes could act as genomic tuning knobs because the intrinsic variation in their junk DNA content would indirectly result in a variation in gene expression for many genes across the genome and phenotypic traits these genetic products participate on (Diaz-Castillo 2015).

Remarkably, that *Y*/*W* chromosomes could promote phenotypic heterogeneity even in the absence of further genetic changes in genes coding sequences or their direct regulatory motives suggests *Y*/*W* chromosomes could be considered phenotypic heterogeneity-promoting capacitors (Diaz-Castillo 2015). *Y*/*W* chromosomes capacitor ability is supported by sexual dimorphic traits widely found in metazoan species (Diaz-Castillo 2015). On one side, phenotypic heterogeneity promoted by *Y*/*W* chromosomes could help heterogametic individuals better enduring environmental challenges, and, therefore, disperse further than homogametic individuals (Diaz-Castillo 2015). Indeed, heterogametic sex-biased dispersal is well documented in metazoans (Clobert et al. 2012; Dobson 2013; Greenwood 1980; Petit and Excoffier 2009). On the other side, *Y*/*W* chromosomes capacitor role would allow for a sexually dimorphic accumulation of cryptic genetic variation that is noticeable under certain conditions and contributes to the genetic diversification of natural populations (Diaz-Castillo 2015). Indeed, in metazoans, evolution and conditional response of genetic elements whose expression is specific or higher in heterogametic individuals tend to be faster than for genetic elements with specific or higher expression in homogametic individuals or with sexually unbiased expression (Assis et al. 2012; Ellegren and Parsch 2007; Mank et al. 2007; Parsch and Ellegren 2013; Gallach et al. 2011; Jiang et al. 2011; Mank 2009; Meisel 2011; Singh and Artieri 2010; Wyman et al. 2010, 2012).

The accumulation and accentuated variation of junk DNA elements in heterochromatic compartments is not exclusive of *Y*/*W* chromosomes. Non-sexually dimorphic chromosome compartments like autosomal centromeres or telomeres are also almost completely formed by junk DNA (Dimitri et al. 2005). Furthermore, heterochromatic junk DNA repositories other than *Y*/*W* chromosomes are also known to be prone to vary in size even within natural populations or laboratory stocks (Cohen et al. 2005; Cohen and Segal 2009; Gamperl et al. 1982; Halfer 1981; Lyckegaard and Clark 1991; Nova et al. 2002; Singh et al. 1980). Consequently, it is possible that non-sexually dimorphic repositories of heterochromatic junk DNA could also act as genomic tuning knobs, and, because of that, phenotypic heterogeneity-promoting capacitors. The confirmation of the capacitor ability of non-sexually dimorphic repositories of heterochromatic junk DNA could be addressed by studying differences in capacitance-derived traits for species with genomes that differed in their overall amount of heterochromatic junk DNA. However, this might be a complex task at the present moment. Junk DNA elements are very diverse (Palazzo and Gregory 2014), and the decision on how much of a genome could be considered heterochromatic junk DNA should be made based on both known DNA sequence and chromatin structure. Despite much celebrated claims, the sequencing of no eukaryotic genome is yet complete since large repositories of heterochromatic junk DNA are still misrepresented (Elliott and Gregory 2015). Also, up until now, there has been a major preference for the sequencing of small genomes with limited junk DNA fractions (Gregory 2005; Peterson et al. 2009). Therefore, neither heterochromatic junk DNA is well represented in current genome annotations, nor these are representative of the natural spectrum for the overall amount of heterochromatic junk DNA within genomes. Independent sources of evidence exist for genome size variation being mostly caused by junk DNA variation (Elliott and Gregory 2015; Palazzo and Gregory 2014), and directly proportional to genome size (Oliver et al. 2007). Thus, an alternative for the study of heterochromatic junk DNA variation-based trends could rely on the use of genome size as a crude approximation for the species variation in the amount of junk DNA within heterochromatic compartments.

In this Synthesis, I hypothesize on the nature of expected trends for the spatiotemporal and evolutionary dynamics

of natural populations for species with different amounts of heterochromatic junk DNA within their genomes (section I), look for evidence supporting the existence of these trends in the Drosophilidae family (section II), and briefly discuss the effect junk DNA-based differences in evolutionary capacitance might have on other aspects of species dynamics such as extinction and the formation of grouped ecological structures (section III). It is important to highlight that I will be referring to junk DNA in a very liberal way because the most relevant aspect here is the accumulation and accentuated variation in quantity of diverse junk DNA elements in heterochromatic repositories, i.e., mostly highly repetitive DNA and transposable elements. Also, here I use the expression junk DNA variation-based evolutionary capacitance to refer to evolutionary capacitance promoted by the variation in the amount of junk DNA within heterochromatic repositories, and not to the variation in the type of junk DNA elements within these repositories in particular, or whole genomes in general. For the sake of brevity and focus, the interplay between evolutionary capacitance, junk DNA elements diversity within and between species, and other aspects highly relevant such as the mechanisms and forces causing small or large variation in the amount of genomic junk DNA will be addressed independently.

Hypothetical Effect of Junk DNA Variation-Based Evolutionary Capacitance on the Spatiotemporal and Evolutionary Dynamics of Natural Populations

Figure 1 illustrates inferences made for the connection between non-sexually dimorphic heterochromatic junk DNA effect as genomic tuning knobs and natural populations spatiotemporal and evolutionary dynamics for species with genomes containing different amounts of heterochromatic junk DNA. These inferences build on trends on capacitance-driven traits putatively dependent on *Y*/*W* chromosomes acting as genomic tuning knobs (Diaz-Castillo 2015), and assume that genomes with larger amounts of heterochromatic junk DNA could be better phenotypic heterogeneity-promoting capacitors than genomes with smaller amounts of heterochromatic junk DNA. Such differences in evolutionary capacitance would be translated in natural populations for species with junk DNA-enriched large genomes showing enhanced dispersal and random drift of cryptic genetic variation when compared with junk DNA-impoverished small genomes.

Enhanced dispersal for species with junk DNA-enriched large genomes could result in them being organized in widespread lowly dense populations prone to split into non-overlapping subpopulations. Despite such structure could be expected to promote fast genetic differentiation of pseudo-isolated subpopulations, the enhanced dispersal of subpopulation individuals might permit occasional inter-subpopulation contacts that mitigated their genetic differentiation. On the other hand, a limited dispersal for species with junk DNA-impoverished small genomes would result in them being organized in narrower and denser populations. Also, because genetic variation would be more often phenotypically distinguishable from the narrow spectrum of phenotypic heterogeneity dependent on heterochromatic junk DNA variation for species with junk DNA-impoverished small genomes than for species with junk DNA-enriched large genomes, it would be expected that the amount of genetic variation that could contribute to the genetic differentiation of subpopulations would be comparatively larger in the former than in the latter. Consequently, differences in junk DNA variation-based evolutionary capacitance could be reflected by differences in the spatiotemporal structure of natural populations for species with different amounts of heterochromatic junk DNA, and comparatively faster genetic diversification of natural populations for species with junk DNA-impoverished small genomes than for species with junk DNA-enriched large genomes.

Following these predictions, the direct confirmation for junk DNA variation-based evolutionary capacitance effect on the spatiotemporal and evolutionary dynamics of natural populations would require relating genome size variation as proxy for the junk DNA content of heterochromatic repositories and proxies for natural populations spatiotemporal distribution and genetic diversification. The use of genome size as proxy for heterochromatic junk DNA is not exempt of problems though, since genome size is also known to correlate with other traits that can have an effect on the geographic distribution of natural populations. On one side of the spectrum, species with small genomes have been shown to very commonly participate in tight heterospecific ecological associations, i.e., symbiosis, parasitism, or commensalism (Moran and Bennett 2014; Blaxter and Koutsovoulos 2015; Wolf and Koonin 2013). Thus, the spatiotemporal distribution of species with junk DNA-impoverished small genomes could indeed reflect the abilities of the species they associate with more than their own. For example, the *Drosophila melanogaster* species subgroup is formed by nine species with small genomes (Bosco et al. 2007; Gregory and Johnston 2008; David et al. 2007). Seven of these species are highly specialized and their spatiotemporal distribution restricted by these specializations (David et al. 2007). The fact that *D. melanogaster* and *D. simulans*, the other two species of the subgroup, are cosmopolitan questions which lifestyle is ancestral to the melanogaster species subgroup (David et al. 2007). However, since *D. melanogaster* and *D. simulans* are human commensals,

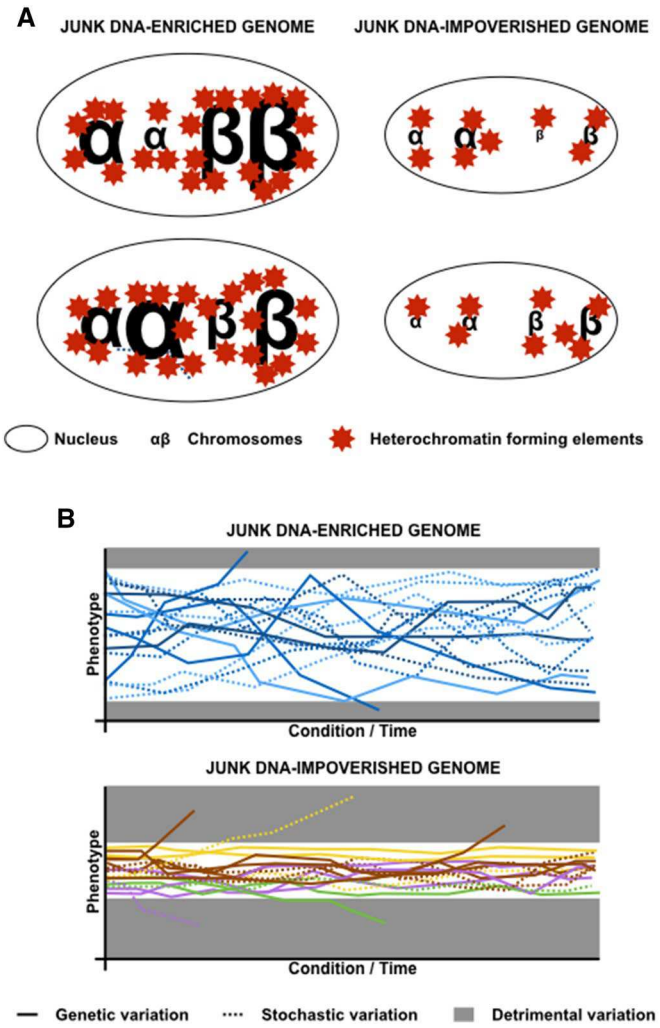

Fig. 1 Hypothetical spatiotemporal and evolutionary dynamics for species with different amounts of heterochromatic junk DNA in their genomes. **a** Cartoon symbolizing the assortment of heterochromatin forming elements in nuclei of individuals with different amounts of junk DNA. *Chromosome font size* represents heterochromatic junk DNA content. The variation in the overall amount of heterochromatic junk DNA is larger for species with junk DNA-enriched genomes than for species with junk DNA-impoverished genomes. Since junk DNA variation in heterochromatic repositories might affect chromatin compaction across genomes, differences in the overall amount of junk DNA would result in larger gene expression heterogeneity across genomes and phenotypic heterogeneity for species with junk DNA-enriched genomes. **b** Charts symbolizing the spatiotemporal dynamics for an ideal phenotype in individuals from species with different amounts of junk DNA. *Lines* represent phenotypic dynamics for single individuals. *Lines with different colors* represent phenotypic dynamics for divergent subpopulations. *Solid or dashed lines* represent phenotypic variation caused by direct genetic changes in genes coding sequence or regulatory motives (genetic), or differences in chromatin compaction promoted by the genomic tuning knob effect of heterochromatic junk DNA repositories (stochastic), respectively. *Grey boxes* represent detrimental phenotypes. Individuals reaching detrimental phenotypes stop contributing to populations. Individuals of species with junk DNA-enriched genomes will be more phenotypically heterogeneous, permitting better coping with environmental changes, and therefore, a better fit with their environment that is represented as a reduction in detrimental phenotypes. **c** Cartoon symbolizing geographic distributions of two species with different amounts of junk DNA. *Dotted lines* symbolize variation in an abiotic factor, i.e., temperature or altitude. *Circles* and *triangles* represent individuals of species with junk DNA-enriched and -impoverished genomes, respectively. Phenotypes for species with junk DNA-enriched genomes are more heterogeneous permitting better coping with environmental changes and therefore further dispersal, which causes populations being more dispersed and less dense. Phenotypes for species with junk DNA-impoverished genomes are less heterogeneous, which might limit individual dispersal, and, consequently promote larger population density. In *panels* **b** and **c**, *shades of blue* symbolize reduced subpopulation genetic differentiation for species with junk DNA-enriched genomes, whereas *different colors* symbolize enhanced subpopulation genetic differentiation for species with junk DNA-impoverished genomes. (Color figure online)

their cosmopolitan distributions are most probably derived from their specific association with humans (Lachaise and Silvain 2004). On the other side of the spectrum, very large genomes have been associated with developmental variations that could limit their dispersal. For example, salamanders with junk DNA-enriched very large genomes tend to be obligate neotenes and, because of that, restricted to aquatic environments (Gregory 2002, 2003; Sclavi and Herrick 2015). Thus, the study of the association between genome size and species geographic distributions alone might be inadequate to seek confirmation for the existence of evolutionary capacitance differences between species with disparate amounts of heterochromatic junk DNA in their genomes.

Study of the Association Between Genome Size Variation and Genetic Diversification for the Drosophilidae Family

Interestingly, a negative correlation has been observed widespread in eukaryotes between genome size and the number of species per taxon or speciosity (Knight et al. 2005; Kraaijeveld 2010; Mank and Avise 2006; Olmo 2006; Sclavi and Herrick 2015; Smith and Gregory 2009; Vinogradov 2003). Such trend could lend some support to the possibility that genetic differentiation leading to speciation was more accentuated for species with junk DNA-impoverished small genomes than for species with junk DNA-enriched large genomes. However, speciosity is not only affected by speciation rates but also extinction rates, and junk DNA accumulation has been suggested to be detrimental (Vinogradov 2003, 2004). Thus, whether evolutionary capacitance differences contribute to the variation in natural population genetic diversification leading to speciation that explained negative correlations between genome size and speciosity required further evidence.

The Drosophilidae family is ideal to study the potential association of variation in junk DNA-based genome size and proxies for the process of genetic differentiation leading to speciation. First, genome size variation in the Drosophilidae family seems to be mostly caused by variation in the overall amount of junk DNA (Bosco et al. 2007; Drosophila 12 Genomes et al. 2007). Second, extensive work has been done to resolve the phylogeny of this very populous family (Bächli 2015; Markow and O'Grady 2005; Russo et al. 2013; Throckmorton 1975; van der Linde et al. 2010; Yassin 2013). Finally and most importantly, an extensive literature also exists for the characterization of the genetic differentiation accumulated between Drosophilidae species since they diverged, and the effect this genetic differentiation might have for their reproductive isolation and speciation (Coyne and Orr 1989, 1997; Yukilevich 2012).

The Animal Genome Size Database includes data for 95 Drosophilidae species (Gregory 2015). Since different genome size determination strategies have particular biases, I proceed to analyze separately data from the two studies that contributed genome size data for more Drosophilidae species to the Animal Genome Size Database, i.e., Bosco and Gregory datasets after their respective first authors (Bosco et al. 2007; Gregory and Johnston 2008). Genome size data for the 18 species represented in both datasets shows a considerably good correlation (Spearman rho = 0.84), suggesting methodological biases might be a minor limitation in fore coming analyses. These two datasets also differ in the number and phylogenetic distribution of species they include. Gregory dataset includes almost twice as many species than Bosco dataset, but their phylogenetic distribution is narrower in the former than in the latter (Fig. 2). These differences result in genome size being on average smaller and less variable for Gregory dataset (Bosco: N = 36, mean = 0.24, coefficient of variation = 0.30; Gregory: N = 64, mean = 0.21, coefficient of variation = 0.20). Since genome size in the Drosophilidae family tends to be considerably small (Gregory and Johnston 2008), any other factor that limited analytical power could considerably difficult the quest for genome size-based trends. Conventional wisdom would suggest that the largest dataset would offer more analytical power, and yet, Bosco dataset analyses showed clearly significant trends, but Gregory dataset analyses did not. This difference between datasets underscores the key importance fair phylogenetic sampling has for certain analyses. Although results are provided for the analyses with both datasets (Table 1), the description that follows is based on the results obtained from the analyses of Bosco dataset.

The analyses here presented were based on the use of the "species group" level as reference taxon to calculate speciosity mostly because classification uncertainties seem to be more accentuated above the species group level (Markow and O'Grady 2005; Throckmorton 1975; Russo et al. 2013; van der Linde et al. 2010; Yassin 2013), previous analyses of genome size variation in the Drosophilidae family showed that genome size varies more between species groups than between upper or lower taxonomic levels (Gregory and Johnston 2008), and species within species groups often participate in interspecific crosses which are ideal for estimating the genetic differentiation accumulated between two species and its effect on their reproductive isolation (Allaby 2003). Furthermore, genome size and species group speciosity correlation in the Drosophilidae family is negative and significantly different from correlations expected by chance using phylogenetically corrected Monte Carlo simulations and the commonly used threshold of significance (P = 0.05) (Fig. 3; Table 1), which agree with the aforementioned trend found widespread in eukaryotes

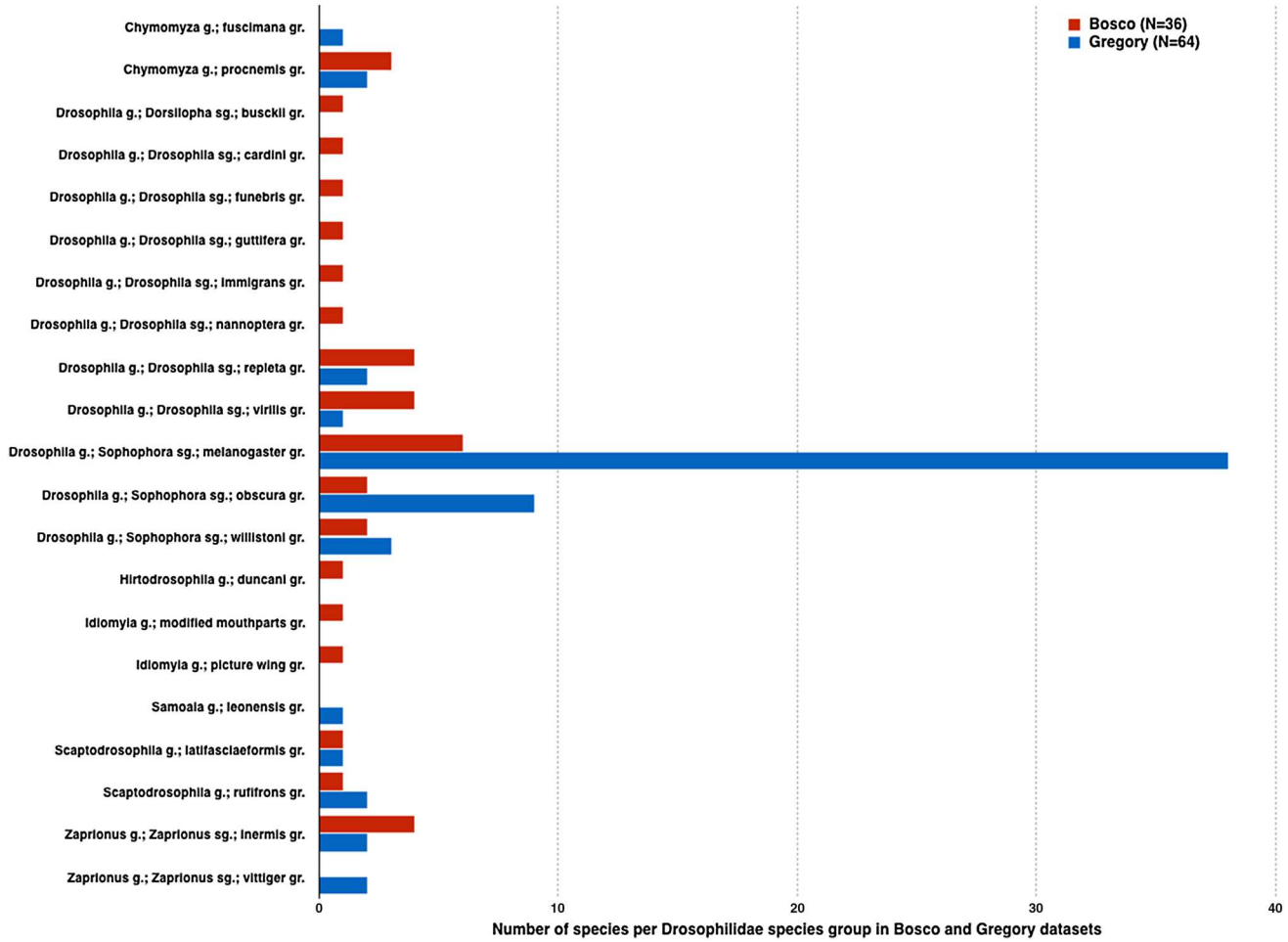

Fig. 2 Species group assortment of Drosophilidae species listed in Bosco and Gregory datasets according to TaxoDros. Genome size data for Drosophilidae species originally produced by Bosco and Gregory were obtained from the Animal Genome Size Database (Gregory 2015; Gregory and Johnston 2008; Bosco et al. 2007). Names and classification of species were verified using TaxoDros (Bächli 2015). Species group in the *y-axis* are arranged alphabetically and not according to their phylogenetic relationship. g., genus; sg., subgenus; gr., species group

(Knight et al. 2005; Kraaijeveld 2010; Mank and Avise 2006; Olmo 2006; Sclavi and Herrick 2015; Smith and Gregory 2009; Vinogradov 2003).

The use of interspecific crosses for species that have diversified enough to be distinguishable but are able to at least partially mate with each other is ideal to characterize the process of speciation in action. If genetic differentiation leading to speciation for species with junk DNA-impoverished small genomes was comparatively faster because of genetic variation is more often phenotypically distinguishable for selective forces, it would be expected that genome size and genetic differentiation measures obtained from interspecific crosses negatively correlated. Here, I consider five measures for genetic differentiation obtained from Drosophilidae interspecies crosses compiled at the Drosophila Speciation Patterns Database (Yukilevich 2012): Nei's *D*, premating isolation index, complete and incomplete postzygotic isolation indexes, and allopatric/sympatric semiquantitative classification. Nei's *D* is a direct estimation for the genetic differentiation accumulated between two species based on per locus allele frequencies (Nei 1972). The premating isolation index quantifies reproductive isolation between two species based on their ability to produce interspecific matings (Coyne and Orr 1989). The postzygotic isolation index quantifies reproductive isolation between two species based on the viability and the fertility of their hybrid progeny (Coyne and Orr 1989). Here, two postzygotic isolation indexes are under consideration, i.e., complete and incomplete, depending if the viability and/or the fertility of the hybrid progeny is completely or incompletely reduced (Yukilevich 2012). Thus, Nei's *D* represents a direct estimation of the genetic differentiation accumulated between two species, whereas premating and postzygotic isolation indexes represent the

Table 1 Covariation of genome size and genetic differentiation proxies for the Drosophilidae family

Genetic differentiation proxy	Genome size dataset	N	Observed Spearman rho	Simulated Spearman rho [Minimum/5th percentile/95th percentile/Maximum]	P_{upper}/P_{lower}
Species group speciosity	Bosco	36	−0.47	−0.71/−0.43/0.43/0.72	0.9688/0.0312
Nei's *D*	Bosco	34	−0.44	−0.77/−0.38/0.37/0.74	0.9740/0.0260
Premating isolation index	Bosco	34	−0.59	−0.77/−0.38/0.37/0.67	0.9969/0.0031
Complete postzygotic isolation index	Bosco	16	−0.64	−0.82/−0.53/0.56/0.74	0.9735 0.0341
Partial postzygotic isolation index	Bosco	18	−0.62	−0.80/−0.51/0.50/0.73	0.9814/0.0196
Allopatry (0)/sympatry (1)	Bosco	34	−0.41	−0.74/−0.38/0.37/0.85	0.9656/0.0352
Species group speciosity	Gregory	64	−0.23	−0.55/−0.33/0.32/0.55	0.8918/0.1082
Nei's *D*	Gregory	112	−0.34	−0.39/−0.18/0.18/0.39	0.9985/0.0015
Premating isolation index	Gregory	104	0.04	−0.43/−0.19/0.19/0.43	0.3650/0.6351
Complete postzygotic isolation index	Gregory	42	−0.18	−0.80/−0.35/0.35/0.77	0.7895/0.2106
Partial postzygotic isolation index	Gregory	60	−0.23	−0.57/−0.29/0.29/0.70	0.9011/0.0989
Allopatry (0)/Sympatry (1)	Gregory	118	0.15	−0.41/−0.18/0.18/0.42	0.0909/0.9095

Nei's *D*, premating and postzygotic isolation indexes, and allopatric/sympatric classification were obtained from Drosophila Speciation Patterns database, whereas species group speciosity was calculated according to TaxoDros (Bächli 2015; Yukilevich 2012). Genome size data originally produced by (Bosco et al. 2007) and (Gregory and Johnston 2008), were retrieved from the Animal Genome Database (Gregory 2015). Simulated Spearman rhos for the covariation of genome size and genetic differentiation proxies were obtained after randomly rearranging genetic differentiation proxies data 10,000 times (see "Materials and Methods" section for further details). P_{upper} and P_{lower} values represent the fraction of random simulations that result in Spearman rho larger or equal, and lower or equal than observed ones, respectively

effect this genetic differentiation can have on their reproductive isolation. As hypothesized, the correlation between genome size and Nei's *D* or isolation indexes was in all cases negative and significantly different from correlations expected by chance using phylogenetically corrected Monte Carlo simulations and the commonly used threshold of significance (P = 0.05) (Fig. 3; Table 1).

The speculative scenario inferred for the spatiotemporal and evolutionary dynamics of species with different amounts of heterochromatic junk DNA in their genomes upon its ability to promote evolutionary capacitance also suggests that the faster genetic differentiation of species with junk DNA-impoverished small genomes might often occur in the absence of geographic isolation. The Drosophila Speciation Patterns Database classifies interspecific crosses as sympatric or allopatric depending on the overlap or lack of it of the current geographic distributions of the parental species (Yukilevich 2012). If such distributions were representative for the geographic distribution these species had during most of their divergence, it would be expected that species with junk DNA-impoverished small genomes would abound among parental species for sympatric interspecific crosses. Agreeing with such expectation, the correlation between genome size and sympatry/allopatry semiquantitative classification is negative and significantly different from correlations expected by chance using phylogenetically corrected Monte Carlo simulations and the commonly used threshold of significance (P = 0.05) (Fig. 3f; Table 1).

In summary, the covariation of genome size and measures for genetic differentiation obtained from Drosophilidae interspecific crosses are consistent with predictions I made based on the potential effect of the overall amount of heterochromatic junk DNA within genomes on evolutionary capacitance. Drosophilidae species with junk DNA-impoverished small genomes seem to show an enhanced genetic differentiation leading to speciation compared to species with junk DNA-enriched genomes. Also, although the use of species geographic distributions have important caveats I already discussed, the present analyses are consistent with the possibility that genetic diversification of species with junk DNA-impoverished small genomes occurred even in the absence of geographic isolation.

Junk DNA Variation-Based Evolutionary Capacitance Effect on Extinction and the Formation of Grouped Ecological Structures

In vertebrates and plants, it has been shown that species with large genomes tend to abound within lists of endangered species, which has been used to argue that junk DNA accumulation might be detrimental (Vinogradov 2004, 2003). Thus, the negative correlation between genome size and speciosity documented here and in the literature could reflect not a difference in speciation rate but in extinction rate, or even both (Fig. 3a; Table 1) (Knight

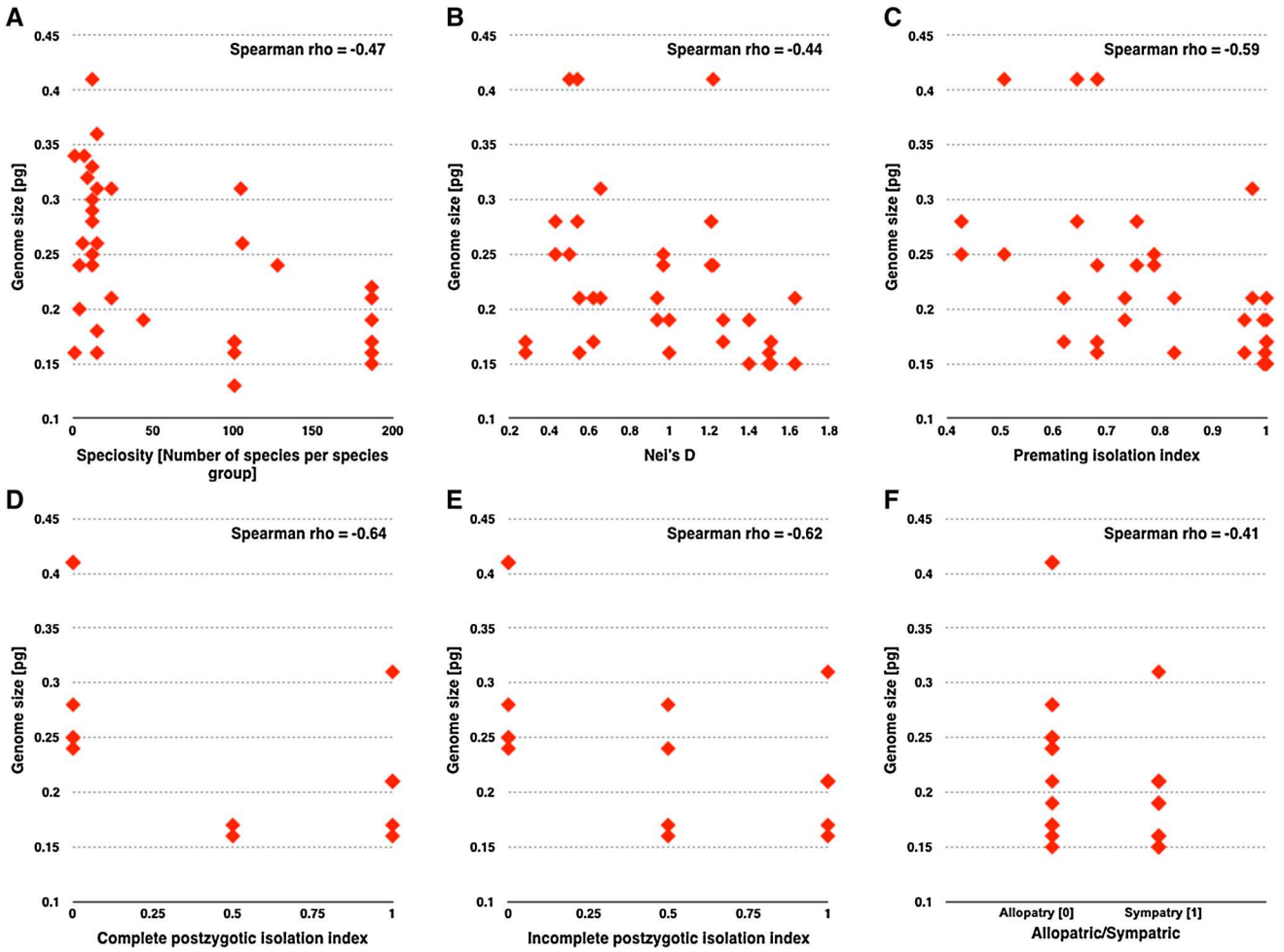

Fig. 3 Covariation of genome size and genetic differentiation proxies for the Drosophilidae family. Genome size data originally produced by Bosco and Gregory were obtained from the Animal Genome Size Database (Gregory 2015; Gregory and Johnston 2008; Bosco et al. 2007). **a** Species group speciosity was calculated according to TaxoDros (Bächli 2015). **b–f** Nei's *D*, premating and postzygotic isolation indexes and allopatric/sympatric classification for interspecific crosses were obtained from the Drosophila Speciation Patterns Database (Yukilevich 2012). Covariation between genome size and genetic differentiation proxies were quantified using Spearman rho

et al. 2005; Kraaijeveld 2010; Mank and Avise 2006; Olmo 2006; Sclavi and Herrick 2015; Smith and Gregory 2009; Vinogradov 2003). However, the potential contribution of heterochromatic junk DNA variation for evolutionary capacitance I here hypothesized permits speculating with extinction trends for species with different junk DNA-based genome sizes that do not agree with the idea that junk DNA accumulation were detrimental, and, consequently, species with larger genomes more susceptible to extinction.

If the accumulation of junk DNA truly promoted a phenotypic heterogeneity that permitted better enduring environmental challenges, it would be expected that species with junk DNA-enriched large genomes were particularly resilient, i.e., able to endure environmental challenges. Interestingly, species with junk DNA-enriched genomes such as lungfishes, cockroaches, or the bacteria *Deinococcus radiodurans* are paramount examples for their resilience to multiple extreme environmental conditions (Joss 2006; Kraaijeveld 2010; Lee et al. 2006; Makarova et al. 2001; National Research Council 2007). However, I also hypothesized that species with junk DNA-enriched genomes might be organized in lowly dense populations prone to fragmentation, and these are traits with a considerable weight for a species to be considered under a high risk of becoming extinct (IUCN Species Survival Commission 2001). Thus, although species with junk DNA-enriched large genomes might be inherently resilient because the abundant heterochromatic junk DNA promotes phenotypic heterogeneity that allow them to cope with variable environments, their widespread population structure might make them susceptible to other factors that contributed to reduce their already

low population density below sustainability limits. For example, the Mexican axolotl or *Ambystoma mexicanum*, a key reemerging biomedical model system mostly because of its ability to regenerate several body parts and with a huge genome, has become very rapidly virtually extinct because of the abrupt deterioration in recent decades of its aquatic environment in Xochimilco, Mexico (Voss et al. 2009, 2015).

Also, limitations in phenotypic heterogeneity-based evolutionary capacitance for species with junk DNA-impoverished small genomes might result in them being less resilient. In fact, past and present examples exist for sudden collapses of very diversified taxa with small genomes. Non-avian dinosaurs, one of the most celebrated examples for a catastrophic decline, have been presumed to carry junk DNA-impoverished genomes when compared with some of the taxa that outlived them (Organ et al. 2007, 2009). Nowadays, particularly refractory to the identification of simple satisfactory explanations is the case of the sudden widespread collapse of domesticated colonies of the honey bee *Apis mellifera*, a species with a considerably junk DNA-impoverished small genome (Barron 2015; Gregory 2015; Kapheim et al. 2015; Perry et al. 2015; Staveley et al. 2014; The Honeybee Genome Sequencing Consortium et al. 2006). Furthermore, the limited resilience for species with junk DNA-impoverished small genomes might be even more critical in the current context of global increase of temperatures. Genome-wide chromatin compaction seems to be reduced when very early embryogenesis occurs in warmer temperatures (Biamonti and Vourc'h 2010; Elgin and Reuter 2013; Hartmann-Goldstein 2009). Since chromatin compaction might promote gene expression heterogeneity (Kaern et al. 2005; Raj and van Oudenaarden 2008; Raser and O'Shea 2005), it would be expected that any factor contributing to a general reduction in genome-wide chromatin compaction resulted in a reduction in gene expression heterogeneity across genomes. Following upon the logic here proposed, a temperature-based reduction in gene expression noise across genomes would be manifested also as a reduction in phenotypic heterogeneity, evolutionary capacitance, and resilience, and, therefore, an increase in the susceptibility for sudden declines. Although temperature-dependent reduction in evolutionary capacitance would be expected to affect all species, it would hit harder to species with an already intrinsically low evolutionary capacitance, such as those with junk DNA-impoverished small genomes. The recent connection established between global warming and the decline in bumblebee species of the genus *Bombus* might illustrate the synergistic pervasive effect of junk DNA-impoverishment and temperature increases for evolutionary capacitance and with it species sustainability (Carswell 2015; Kapheim et al. 2015; Kerr et al. 2015).

Thus, contrary to what it has been inferred in the past under the assumption that junk DNA accumulation might be detrimental (Vinogradov 2003, 2004), it could be possible that species with junk DNA-impoverished small genomes were even more susceptible to succumb to environmental challenges because their low evolutionary capacitance. Two factors might disguise the potential detrimental effect of having too less junk DNA. On one hand, evolutionary capacitance reduction might increase speciation rate as I proposed in here, and, therefore, regardless of their inherent resilience there would always be more species with junk DNA-impoverished small genomes than with junk DNA-enriched large genomes. On the other hand, the limited dispersal and the enhanced release of genetic variation with potential adaptive value for species with junk DNA-impoverished genomes might cause them being organized in denser populations, but also to establish very specialized adaptive interrelations with abiotic or biotic factors. Indeed, it is increasingly evident the association between junk DNA-impoverished small genomes for species organized in large populations or eusocial structures (Kapheim et al. 2015; Morris et al. 2012; Tsutsui et al. 2008), or participating in specialized heterospecific interactions, i.e., symbiosis, parasitism, commensalism, or domestication (Bennett 1976; Moran and Bennett 2014; Diez et al. 2013; Blaxter and Koutsovoulos 2015; Wolf and Koonin 2013). Since grouped ecological structures could confer resilience to the elements that participate in them (Folke 2006; Peterson et al. 1998; Watson et al. 2015), it could be argued that the enhanced participation of species with junk DNA-impoverished genomes in conspecific or heterospecific group structures could at least partly compensated for their inherent limited resilience.

In summary, based on the potential contribution junk DNA-based evolutionary capacitance might have for environmental resilience, it is possible that the overall amount of heterochromatic junk DNA differences not only resulted in evolutionary capacitance-based differences for speciation and dispersal, but also for species extinction. Species with different amounts of junk DNA might not differ on their probability of becoming extinct, but on the type of perils they would succumb to and their strategies to survive them. Species with junk DNA-enriched large genomes might be intrinsically resilient but also less populous, and therefore, susceptible to situations that reduced their already low population density such as overhunting or drastic deteriorations of their environments. On the other hand, species with junk DNA-impoverished small genomes might be prone to participate in grouped ecological structures that provide them with an emergent resilience, but also intrinsically fragile and therefore susceptible to catastrophic collapses. The possibility that the overall amount of junk DNA within genomes had also an influence on species extinction

dynamics opens the door to use heterochromatic junk DNA as a marker for the type of risks species might be ready to confront and succumb to and improve our understanding of biodiversity dynamics and conservation attempts.

Conclusions

In Diaz-Castillo (2015) and in here, I hypothesized that heterochromatic junk DNA indirectly causes a phenotype heterogeneity that promotes evolutionary capacitance and found very preliminary support for junk DNA variation-based evolutionary capacitance differences between sexes of the same species or between species (Fig. 4) (Diaz-Castillo 2015). Differences in evolutionary capacitance based in the presence/absence of junk DNA-enriched sex-specific chromosomes might be manifested in sexual dimorphisms for dispersal and sex-biased gene expression evolutionary and conditional dynamics (Fig. 4a) (Diaz-Castillo 2015). Differences in evolutionary capacitance between species

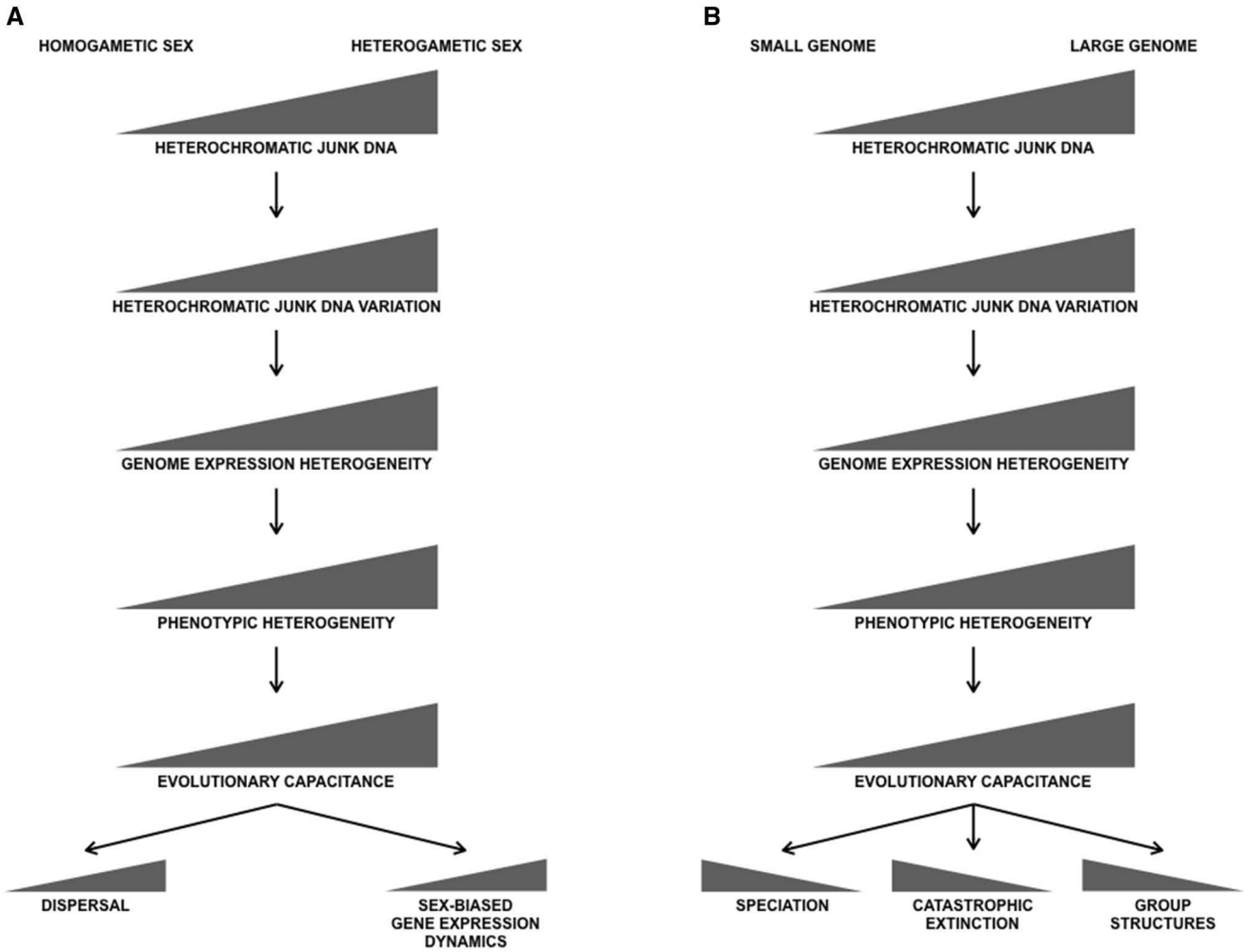

Fig. 4 Junk DNA variation-based evolutionary capacitance for sexes of the same species and different species. See main text for detailed description of these differences and references. The variation in the amount of junk DNA in heterochromatic repositories is larger for individuals with larger amounts of heterochromatic junk DNA than for individuals with smaller amounts of heterochromatic junk DNA. Since junk DNA content variation in heterochromatic repositories acting as a genomic tuning knob might affect chromatin compaction across genomes, the differences in the overall amount of heterochromatic junk DNA might be manifested as larger gene expression heterogeneity across genomes or genome expression heterogeneity, phenotypic heterogeneity, and evolutionary capacitance for individuals with larger overall amount of heterochromatic junk DNA. Considering that chromosomes found in only one sex such as *Y* and *W* tend to be highly enriched in heterochromatic junk DNA, the overall amount in heterochromatic junk DNA would tend to be higher for heterogametic individuals than for homogametic individuals of the same species. Previously, I proposed that the junk DNA variation-based sexual dimorphism in evolutionary capacitance might be manifested as enhanced dispersal and faster sex-biased gene expression dynamics for heterogametic individuals (Diaz-Castillo 2015) (**a**). Here, I propose that species differences in heterochromatic junk DNA variation-based evolutionary capacitance might be manifested as differences for speciation, extinction, and the participation in grouped ecological structures (**b**)

based on the amount of heterochromatic junk DNA within their genomes might be manifested in differences in speciation, dispersal, extinction or the participation in grouped ecological structures (Fig. 4b). The actual scarcity of data for heterochromatic junk DNA enriched elements within "fully" sequenced genomes, or even for species with junk DNA-enriched genomes is a great inconvenient to test further the contribution of heterochromatic junk DNA for evolutionary capacitance. The investment on methodological approaches that permit a fair representation of junk DNA in heterochromatic repositories (Barron et al. 2014; Krsticevic et al. 2015; Richards and Murali 2015), and on patience for painstaking experimentation and data collection for species with junk DNA-enriched large genomes will be crucial for further characterization of junk DNA contribution to evolutionary capacitance.

Materials and Methods

Genome size data for species of the Drosophilidae family were obtained from the Animal Genome Size Database (Gregory 2015). Measures for species genetic differentiation and its effect on reproductive isolation originally obtained from Drosophilidae interspecific crosses were retrieved from the Drosophila Speciation Patterns Database (Yukilevich 2012). Species richness or speciosity was estimated according to the Drosophilidae taxonomy maintained in TaxoDros (Bächli 2015). Names and phylogeny of the species listed in the Animal Genome Size Database and the Drosophila Speciation Patterns Database were verified using TaxoDros (Bächli 2015; Gregory 2015; Yukilevich 2012). Species in the Animal Genome Database and the Drosophila Speciation Patterns Database were discarded if their name did not match valid names, or were considered subspecies in TaxoDros (Bächli 2015; Gregory 2015; Yukilevich 2012).

Analyses were performed independently for genome size data originally produced in two different studies, Bosco and Gregory datasets hereinafter (Online Resource 1) (Bosco et al. 2007; Gregory and Johnston 2008). To study the covariation of genome size and speciosity, for each species in Bosco and Gregory datasets, genome size data was paired with the speciosity of the species group they belong to, i.e., the number of species classified in the species group in question according to TaxoDros (Online Resource 1). To study the covariation of genome size and genetic differentiation measures, I only considered those interspecific crosses listed in the Drosophila Speciation Patterns Database in which the two parental species belong to the same species group, and for both species there is genome size data either in Bosco dataset or in Gregory dataset—17 and 59 interspecific crosses, respectively (Online Resource 2). Here, I consider five measures related with the process of species genetic differentiation obtained from Drosophilidae interspecies crosses (Yukilevich 2012). Nei's D represents a direct measure for genetic distance between two species based on accumulated allele differences per locus (Nei 1972). The premating isolation index measures reproductive isolation between two species based on their ability to mate (Coyne and Orr 1989). Complete or partial postzygotic isolation indexes measure reproductive isolation between two species based on complete or partial limitations to viability and fertility of their hybrid progeny, respectively (Coyne and Orr 1989). Finally, interspecific crosses are classified as sympatric or allopatric if the current geographic distribution of their parental species does or does not overlap, respectively (Coyne and Orr 1989). Integers 1 and 0 were assigned to sympatric and allopatric interspecific crosses respectively to permit correlational analyses (Coyne and Orr 1989; Yukilevich 2012). For each interspecific cross, genome size data for each parental species was paired with their unique genetic differentiation proxy, i.e., each interspecific cross contributes two data pairs to the final dataset.

The covariation of genome size and speciosity or genetic differentiation measures was quantified using Spearman rho (Fig. 4; Table 1). The statistical relevance of observed Spearman rhos was estimated using phylogenetic corrected Monte Carlo simulations (Table 1). In the case of the covariation of genome size and speciosity, Spearman rho was recalculated after randomly rearranging species group speciosity 10,000 times respecting genome size assortment within each species group. In the case of the covariation of genome size and genetic differentiation measures, Spearman rho was recalculated after randomly rearranging genetic differentiation measures 10,000 times respecting parental genome sizes for each interspecific cross. P_{upper} and P_{lower} were calculated as the fraction of simulations with equal or higher, and equal or lower Spearman rho than observed ones, respectively. Microsoft® Excel® for Mac 2011 was used to process data and perform statistical analyses.

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Compliance with Ethical Standards

Conflict of interest The author declares that he has no conflict of interest.

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