Convergent evolution associated with the loss of developmental diapause may promote extended lifespan in bees

Priscila K. F. Santos[1], Karen M. Kapheim[1]

[1]Utah State University, Logan, UT, 84322, United States of America

Corresponding author: Priscila K. F. Santos (pkfsantos1@gmail.com)

Abstract

Diapause has long been proposed to play a significant role in the evolution of eusociality in Hymenoptera. Recent studies have shown that shifts in the diapause stage precede social evolution in wasps and bees, however, the genomic basis remains unknown. Given the overlap in molecular pathways that regulate diapause and lifespan, we hypothesized that the evolutionary loss of developmental diapause may lead to extended lifespan among adults, which is a prerequisite for the evolution of eusociality. To test this, we compared 27 bee genomes with or without prepupal diapause. Our results point to several potential mechanisms for lifespan extension in species lacking prepupal diapause, including the loss of the growth hormone PTTH and its receptor TORSO, along with a significant overlap between genes for which selection is intensified and those with a known role in aging. Specifically, we observed purifying selection of pro-longevity genes and relaxed selection of anti-longevity genes within the IIS/TOR pathway in species that have lost prepupal diapause. Changes in selection pressures on this pathway may lead to the evolution of new phenotypes, such as lifespan extension and altered responses to nutritional signals, that are crucial for social evolution.

Introduction

The origin of eusociality is one of the major transitions in evolution, because it involves a shift from reproduction at the level of the individual to reproduction at the colony level (Szathmáry and Smith 1995). A typical eusocial colony of ants, bees, or wasps includes a long-lived queen who specializes on egg-laying living alongside her non-reproductive daughters who perform nest maintenance, foraging, and brood care (Wilson 1971). Understanding the selective

pressures shaping this major transition requires investigating the origins of each of its defining features; (1) overlapping generations, (2) reproductive division of labor, and (3) cooperative brood care (Batra 1966; Michener 1969). For example, one of the preconditions for overlapping generations is that nest foundresses (queens) live long enough to overlap with their adult daughters (workers). This likely means that the origins of eusociality were preceded by an extension of lifespan in the solitary ancestors (Michener 1974; Carey 2001). However, the mechanisms by which this lifespan extension evolved are unknown.

A potential source of this lifespan extension could be rooted in the phenomenon of diapause. Diapause is a period of dormancy in insects, in which development and reproduction are suppressed, usually as a way to pass unfavorable periods such as extreme temperatures, drought, or scarcity of resources (Denlinger 2022). In bees, diapause most commonly occurs in larvae following the last molt, a stage referred to as the prepupa (Gauld and Bolton 1988; Santos et al. 2019). However, there is a great deal of variation between bee species, with many bees diapausing as adults, and many skipping diapause altogether (Santos et al. 2019). Recent research indicates that prepupal diapause is the ancestral state in bees, but it has been lost in the ancestor of every clade in which eusociality has evolved (Santos et al. 2019). This pattern has been found in both bees and wasps (da Silva 2021), suggesting that while diapause itself may not be essential for social evolution, its loss during the prepupal stage could be pivotal. One potential link between the loss of prepupal diapause and social evolution is in the lifespan-extending features of diapause.

Many of the molecular pathways that regulate diapause in insects also regulate aging and longevity (Hutfilz 2022). While diapause across various life stages shares common features like reduced metabolism, increased lipid reserves, resistance to extreme environmental conditions, and prolonged lifespan, its physiological regulation varies. In prepupal diapause, there is a halt in development, whereas during adult diapause, there is a cessation in reproduction (Denlinger 2022). The stage during which diapause occurs affects how the insulin/insulin-like signaling and target of rapamycin (IIS/TOR) pathway, known for regulating both diapause and aging, functions. During developmental diapause, it manages nutrient allocation for growth and metamorphosis, while during adult diapause, it allocates nutrients for maintenance and reproduction (Denlinger 2022). This suggests that shifts in the diapause stage could promote changes in pathways regulating aging, allowing for lifespan extension.

One other important difference between prepupal and adult diapause is the opportunity for cellular regeneration. Diapause during development is followed by the renewal of somatic tissues through cell divisions and the process of metamorphosis itself (Tatar and Yin 2001). Metamorphosis allows for a restructuring of the organism's body, which can rejuvenate tissues, with potentially lifespan extending effects (Gilbert 2000; Seifert et al. 2012). However, in adults, most of the tissues are post-mitotic, meaning they cannot undergo cell division to renew themselves, limiting the extent to which lifespan can be extended by diapause alone. Adult insects that go through diapause mitigate its potential negative effects by reducing age-specific mortality through a reduced rate of senescence (Tatar and Yin 2001; Easwaran and Montell 2023). The loss of prepupal diapause associated with adaptations to reduce senescence rate

may be key in promoting the overlap of generations and the evolution of social behavior in bees.

If shifts in diapause stage result in prolonged lifespan, then the convergent loss of developmental diapause in bees should be linked to genomic changes associated with adult lifespan extension. To test this hypothesis, we compared the genomes of 27 bee species with and without prepupal diapause. We looked for signals of positive, purifying, and relaxation of selection, as well as gene family gain and losses linked to the absence of prepupal diapause. Next, we assessed whether genes under selection were enriched for longevity or oxidative stress-related functions. We found that the loss of prepupal diapause might be associated with adult lifespan extension through processes including the loss of the growth hormone PTTH and its receptor TORSO, purifying selection of pro-longevity genes, and relaxed selection of anti-longevity genes in the IIS/TOR pathway.

Results

Genomic data selection and orthogroups identification

Out of the 74 bee genomes with available annotation files and information regarding the diapause stage, 71 genomes exhibited over 80% complete BUSCO genes within their protein-coding sequences. Following the exclusion of species from the same genus sharing identical life-history traits, we retained 27 species for subsequent selection analysis (Figure 1; Table S1).

Orthofinder analysis yielded 2592 single-copy orthogroups across all 27 species. After filtering to retain orthogroups present in all species, but being single-copy in at least 70% of them, we identified and utilized 4173 orthogroups for further analysis.

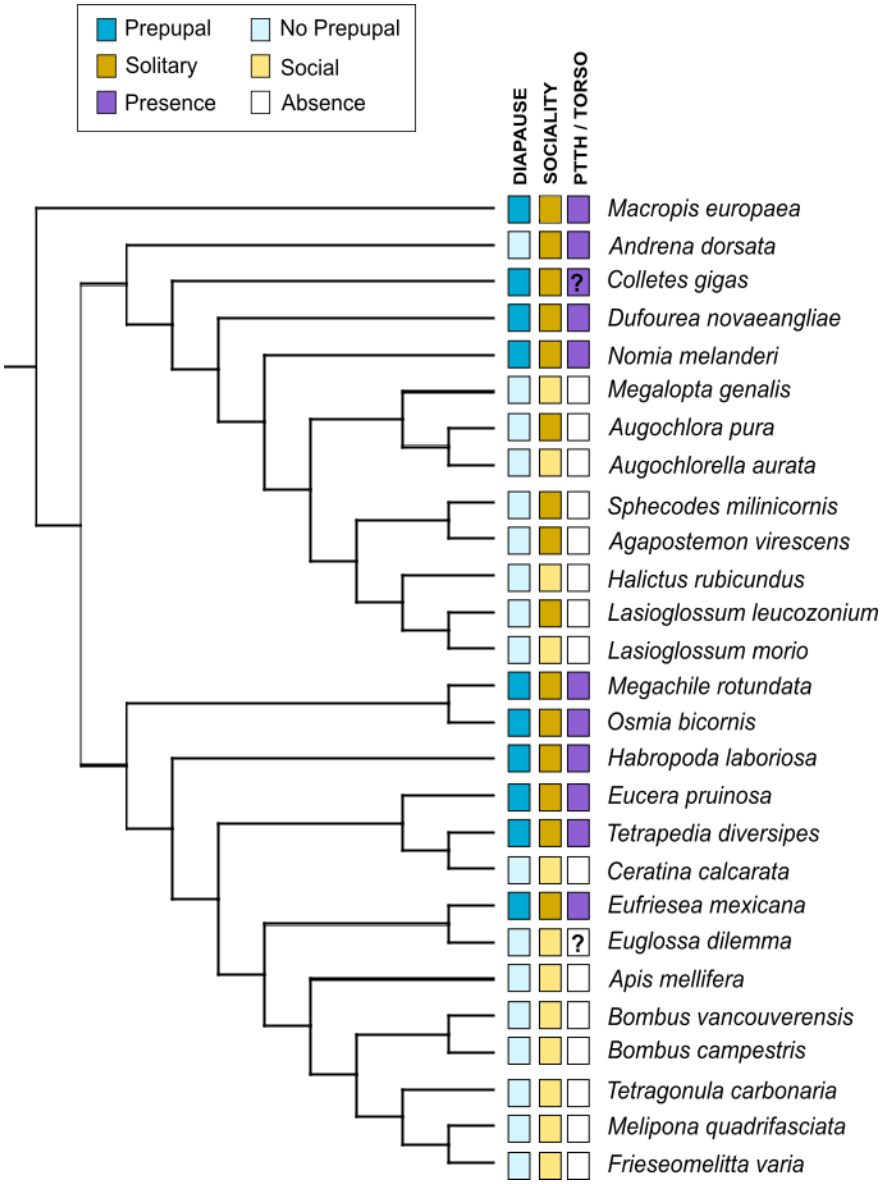

Figure 1: The 27 species included in the molecular evolution analysis and their associated life-history traits. The prepupal diapause stage is depicted in dark blue, while the loss of developmental diapause is represented in lighter blue. Solitary bees are indicated by a stronger

yellow color, and social species are denoted by a lighter yellow. Purple represents the presence of PTTH and its receptor TORSO, while the absence of color indicates orthologs could not be found. The two species marked with "?" had conflicting results regarding the presence of the genes, depending on whether the search was conducted using the genome or the annotated protein-coding genes set as a reference.

Predominance of relaxation of selection linked to the loss of prepupal diapause

The search for convergent evolutionary rate shifts linked to the loss of prepupal diapause using RERconverge resulted in 776 significant orthogroups (Benjamini-Hochberg adjusted p-value < 0.05 after permulation). Among these orthogroups, 283 exhibited positive Rho values, signifying genes undergoing accelerated evolution (attributed to the release of selective constraint or adaptation), whereas 493 showed negative Rho values indicating a decelerated evolutionary rate (increased selective constraint) in species without diapause during developmental stage (Table S2).

Hyphy-aBSREL identified 98 orthogroups where positive selection occurred on a proportion of branches in species lacking diapause in the prepupal stage. However, the significance in most groups is associated with one species (at most three species), suggesting a weak convergent signal of positive selection related to the loss of prepupal diapause (Table S3). Using Hyphy-RELAX, 250 orthogroups were identified under intensification of selection (potentially positive or purifying selection) and 1233 orthogroups under relaxation of selection (Table S3).

Combining results from RERconverge and Hyphy, we categorized orthogroups into groups of interest (see Material and Methods for details): possible positive selection – 267 orthogroups; possible purifying selection – 343 orthogroups; possible relaxed selection – 1046 orthogroups (Figure 2A); and possible intensified selection – 177 orthogroups (Figure 2B). We also classified these orthogroups as having the best evidence of positive selection (49 orthogroups) – those that were significant in at least two of the following: aBSREL, RERconverge with Rho positive values, and intensification of selection in RELAX (Figure 2B and Table 1); or having the best evidence of purifying selection (27 orthogroups) – orthogroups significant in both RERconverge with Rho negative values and intensification of selection from RELAX (Figure 2B; Table 2).

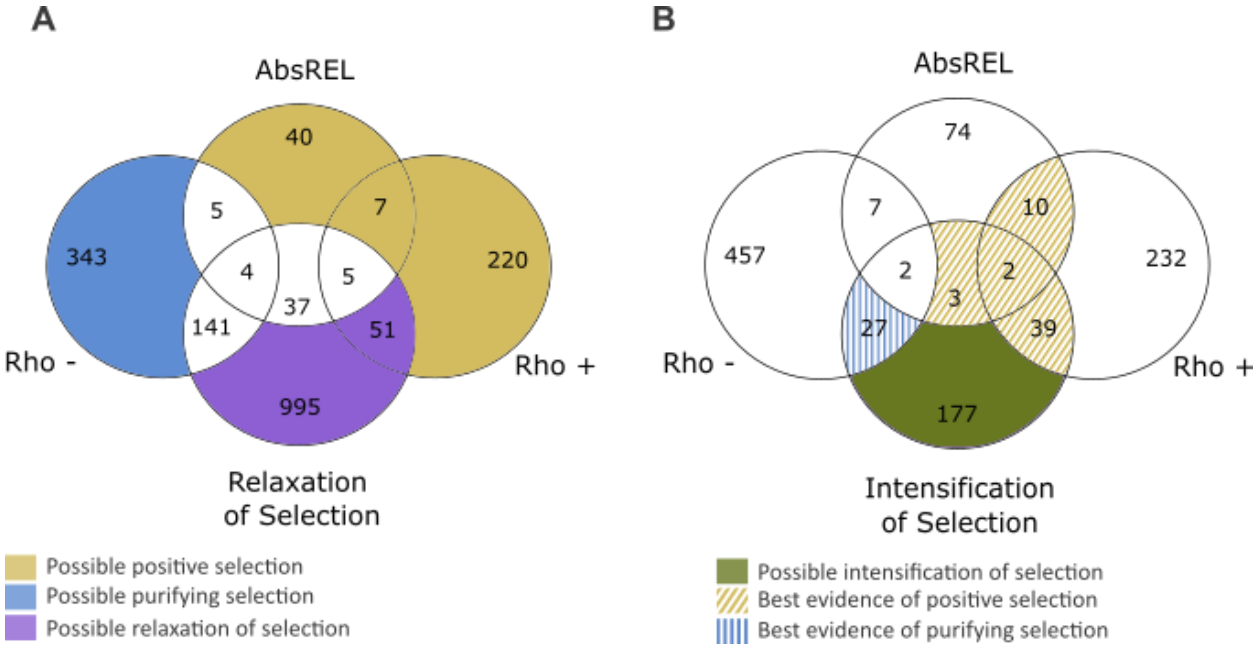

Figure 2: The Venn diagram depicts orthogroups categorized under relaxed (A) or intensified (B) selection, which intersect with genes under positive selection (AbsREL), accelerated evolution

(Rho +), and/or decelerated evolution (Rho -). Different colors denote orthogroups potentially experiencing positive (yellow), purifying (blue), relaxed (purple), or intensified (green) selection, while dashed lines indicate orthogroups with the strongest evidence for positive (yellow) and purifying (blue) selection. Refer to the Material and Methods section for comprehensive classification details.

Table 1: Orthogroups significant in at least two analyses indicating positive selection (intensification of selection, Rho+, and AbsREL), while not significant for deaccelerated selection (Rho -) and relaxation of selection, provide the best evidence of positive selection. The "+" denotes significance for the respective approach, with "Annotation" resulting from blastp against the invertebrate RefSeq database, accessed on November 2023 (O'Leary et al. 2016).

Best evidence of positive selection					
ID	Rho -	Intensification of Selection	Rho +	AbsREL	Annotation
OG0005132		+	+	+	ER membrane protein complex subunit 1
OG0005069		+	+	+	leucine-rich repeat serine/threonine-protein kinase 1
OG0005430		+		+	kinesin-like protein KIF21A
OG0001545		+		+	rho GTPase-activating protein 100F
OG0004149		+		+	E3 ubiquitin-protein ligase UBR5
OG0004348		+	+		protein ST7 homolog
OG0004749		+	+		transcription factor SPT20 homolog [Apis mellifera]
OG0004406		+	+		ER membrane protein complex subunit 3
OG0004926		+	+		uncharacterized protein LOC108001607 isoform X1 [Apis mellifera]
OG0003650		+	+		G1/S-specific cyclin-E1
OG0003667		+	+		tetratricopeptide repeat protein 7B
OG0004654		+	+		INO80 complex subunit D
OG0001279		+	+		protein lin-54 homolog
OG0003654		+	+		Golgi apparatus protein 1
OG0004774		+	+		cyclin-dependent kinase 14
OG0005905		+	+		Krueppel-like factor 6
OG0004721		+	+		epidermal growth factor receptor substrate 15-like 1
OG0003945		+	+		fizzy-related protein homolog
OG0001143		+	+		breast cancer anti-estrogen resistance protein 1
OG0002670		+	+		elongin-C isoform X1 [Apis mellifera]
OG0004851		+	+		TATA-binding protein-associated factor 172
OG0004741		+	+		rho-associated protein kinase 2
OG0005722		+	+		diacylglycerol kinase theta
OG0003659		+	+		protein son of sevenless
OG0005414		+	+		serine/threonine-protein phosphatase 4 regulatory subunit 3
OG0004729		+	+		TOM1-like protein 2
OG0005332		+	+		exocyst complex component 7
OG0005708		+	+		calpain-D-like

OG0005757		+	+		uncharacterized protein C1orf194
OG0005762		+	+		zinc finger MYND domain-containing protein 11
OG0005779		+	+		EARP-interacting protein homolog |protein TSSC1
OG0003248		+	+		dnaJ homolog dnj-5
OG0003324		+	+		uncharacterized protein LOC412655 [Apis mellifera]
OG0002868		+	+		uncharacterized protein LOC412397 [Apis mellifera]
OG0005674		+	+		eIF-2-alpha kinase activator GCN1
OG0001536		+	+		calumenin
OG0003199		+	+		uncharacterized protein LOC100578893 isoform X1 [Apis mellifera]
OG0003227		+	+		set1/Ash2 histone methyltransferase complex subunit ASH2
OG0003204		+	+		histone-lysine N-methyltransferase, H3 lysine-79 specific-like | trichohyalin
OG0004810		+	+		small G protein signaling modulator 3 homolog
OG0004642		+	+		E3 ubiquitin-protein ligase MSL2
OG0005107		+	+		activin receptor type-1
OG0005633		+	+		serine/threonine-protein kinase minibrain
OG0003992		+	+		E3 ubiquitin-protein ligase HECW2
OG0004289			+	+	synaptotagmin 1
OG0005419			+	+	N-terminal kinase-like protein
OG0005795			+	+	E3 ubiquitin-protein ligase TRIM33
OG0004585			+	+	eukaryotic translation initiation factor 3 subunit B
OG0005798			+	+	patatin-like phospholipase domain-containing protein | 1-acylglycerol-3-phosphate O-acyltransferase Pnpla3

Table 2: Orthogroups significant for both intensification of selection and deaccelerated selection (Rho-), while not significant for relaxation of selection, provide the best evidence of purifying selection. The “+” denotes significance for the respective approach, with "Annotation” resulting from blastp against the invertebrate database.

Best evidence of purifying selection					
ID	Rho -	Intensification of Selection	Rho +	AbsREL	Annotation
OG0003941	+	+			uncharacterized protein LOC551214 isoform X1 [Apis mellifera]
OG0001969	+	+			neuroligin 5 isoform X1 [Apis mellifera]
OG0005172	+	+			uncharacterized protein KIAA2013 homolog
OG0000929	+	+			G protein-coupled receptor kinase 1
OG0005376	+	+			solute carrier family 12 member 4
OG0002703	+	+			mitogen-activated protein kinase 15
OG0003858	+	+			armadillo segment polarity protein
OG0001014	+	+			protein zerknuellt 1-like
OG0002754	+	+			dipeptidase 1
OG0002553	+	+			globin-1-like
OG0005042	+	+			thioredoxin reductase-like selenoprotein T homolog CG3887
OG0003676	+	+			protein arginine N-methyltransferase 9
OG0004815	+	+			probable serine/threonine-protein kinase DDB_G0282963 [Apis mellifera]
OG0004859	+	+			importin-7
OG0003382	+	+			uncharacterized protein LOC408957 isoform X1 [Apis mellifera]
OG0002942	+	+			microsomal triglyceride transfer protein large subunit
OG0001556	+	+			mitochondrial carrier protein Rim2 [Apis mellifera]
OG0002013	+	+			COMM domain-containing protein 4
OG0002740	+	+			serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon
OG0004316	+	+			tolloid-like protein
OG0001618	+	+			protein slowmo | PRELI domain containing protein 3A
OG0002630	+	+			A disintegrin and metalloproteinase with thrombospondin motifs 9
OG0004924	+	+			AFG3-like protein 2

OG0003351	+	+			dnaJ homolog subfamily C member 11
OG0003690	+	+			diuretic hormone 44
OG0003333	+	+			uncharacterized protein LOC413052 [Apis mellifera]
OG0004280	+	+			ephrin-B2 [Apis mellifera]

Gene ontology enrichment

Orthogroups exhibiting possible positive and purifying selection were significantly enriched for terms associated with post-translational protein modifications, such as protein phosphorylation (positive and purifying selection) and protein dephosphorylation (positive selection), as well as the regulation of transcription (Figure 3A-B;Table S4). Orthogroups under intensification of selection were enriched for terms related to protein catabolic process, actin cytoskeleton organization, and mitochondrial functions (Figure 3C;Table S4). Enriched terms for orthogroups under relaxed selection included those associated with double-strand break repair, DNA recombination, RNA methylation, autophagy, fatty acid metabolic process, and oxidoreductase activities (Figure 3D;Table S4).

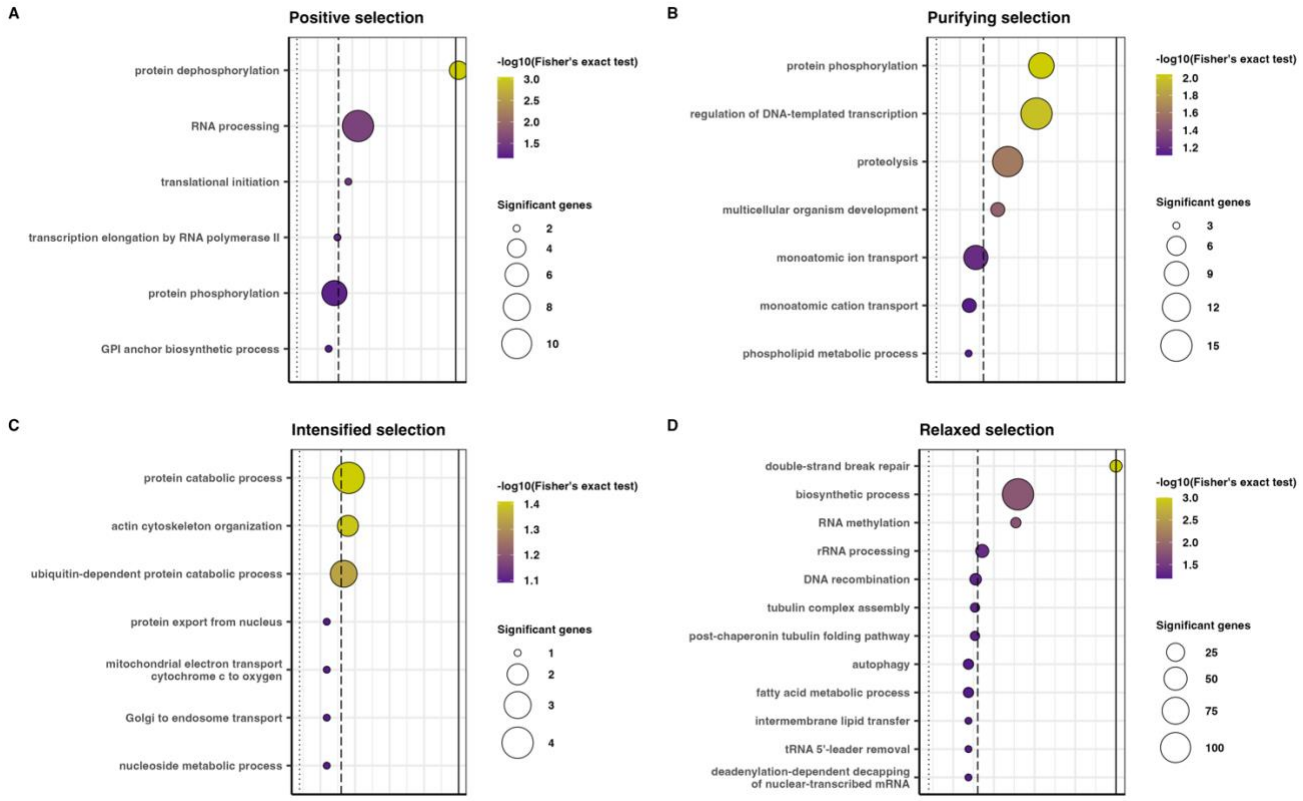

Figure 3: Significant gene ontology enrichment terms ($p < 0.1$ and Annotated genes > 1) in the Biological Processes category. Each plot represents one of the orthogroups classification – possible positive (A), purifying (B), intensified (C), and relaxed (D) selection. P-values are represented by -log(Fisher's exact test) in a color range, with yellow indicating terms with the smallest p-values. Different circle sizes represent the number of significant genes within each term. The three lines represent different p-values cutoffs: dotted = 0.2, dashed = 0.05, and solid = 0.001.

Prepupal diapause loss is a strong predictor of PTTH and torso loss

An analysis of gene family gain and loss revealed that only two orthogroups were lost in the majority of species that do not undergo diapause during development (Table S5). Specifically,

the prothoracicotropic hormone (*PTTH*) gene and its tyrosine-protein kinase receptor torso (*torso*) were absent in all species lacking prepupae diapause, except for *Andrena dorsata*, which has both orthogroups and two copies of the *torso* receptor (Figure 1, Table S5). These losses are followed by positive selection in genes of the EGFR signaling pathway, which is the main pathway producing ecdysone during metamorphosis (Cruz et al. 2020). Notably, *egfr substrate 15* (*egfr15*) and *son of sevenless* (SOS) genes show strong evidence of positive selection (Table 1 and Figure 4).

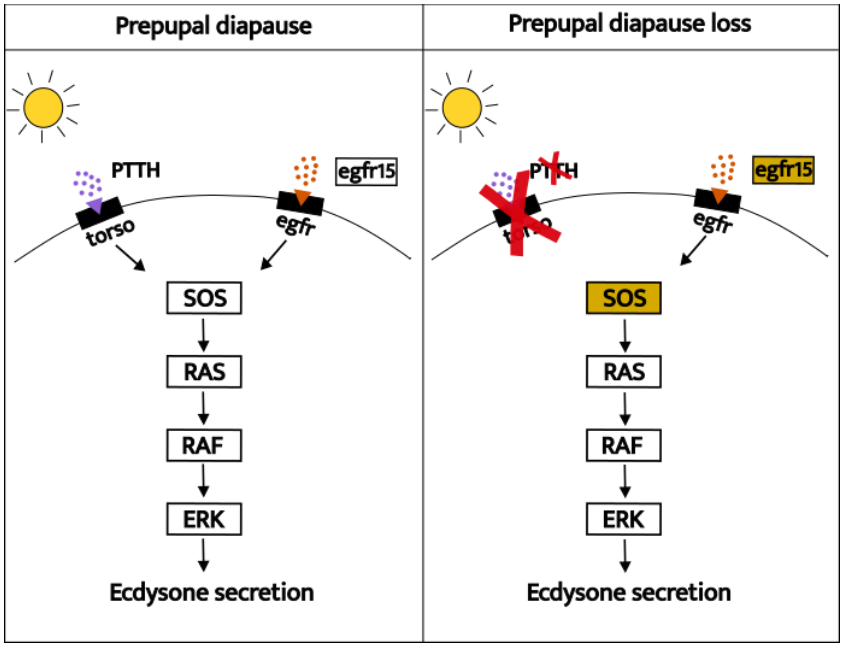

Figure 4: Simplified scheme illustrating two pathways regulating the production and release of ecdysone by the prothoracic gland (PG). The pathway utilizing TORSO as a receptor for PTTH is absent in species that do not undergo prepupal diapause. EGFR15, a substrate of the alternative pathway that uses EGFR as a receptor, is subject to positive selection, along with the *SOS* gene, highlighting the significance of this pathway in species without prepupal diapause.

PTTH and *torso* loss have been previously associated with social behavior in bees. In a previous screening of 22 bee genomes, all 15 of the included social species were missing both *PTTH* and *torso* (Costa et al. 2022). We aimed to discern whether the diapause stage or sociality exerted a stronger influence on gene loss. To address this, we performed PGLS analyses, considering both predictor variables independently and simultaneously (Table S6). When analyzed individually, both diapause stage (Estimate: 0.81, Multiple R-squared: 0.87, p = 2e-13) and sociality (Estimate: 0.48, Multiple R-squared: 0.44, p=8e-05) significantly predicted the presence/absence of *PTTH* and *torso*. However, in the joint analysis, diapause stage is the only significant predictor (Estimates: Diapause(yes) – 0.81, Sociality(solitary) – 0.10; p-values: Diapause – 3e-10, Sociality – 0.1186). Furthermore, the model with both predictors had a slightly higher Multiple R-squared = 0.88 (vs 0.87 including only diapause as predictor), indicating diapause stage is a stronger predictor of *PTTH* and *torso* loss.

Orthogroups under intensification of selection are enriched for lifespan genes

Out of 198 genes related to aging from the GenAge database (Tacutu et al. 2018), we identified 148 among our 4172 orthogroups. We found aging genes among all genes we identified as under possible positive, purifying, intensified, and relaxed selection associated with the loss of prepupal diapause, with the orthogroups under relaxation of selection including the highest number of aging genes (37 out of a total of 1046 genes). However, the intensification of selection group was the only one exhibiting more aging genes than expected by chance (11 out of 177 total; Hypergeometric test: p = 0.048). Many of the aging genes under selection are part

of the IIS/TOR pathway (Figure 5). At least two genes previously described as pro-longevity, and at least five genes described as anti-longevity in *Drosophila* (Tacutu et al. 2018), were identified as under purified and relaxed selection in the current study, respectively (Figure 5 and Table 3).

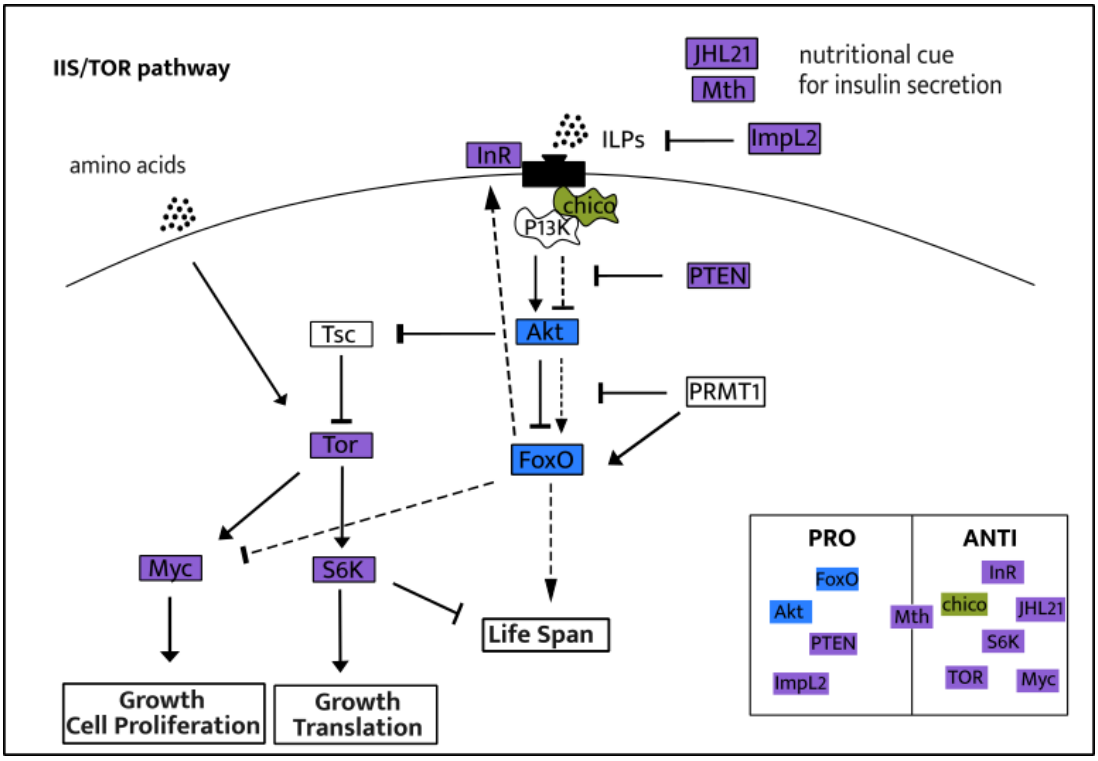

Figure 5: Simplified scheme of IIS/TOR pathway containing genes under selection in the current study. When the insulin pathway is activated (high nutrition), Akt phosphorylates FoxO, preventing its entrance to the nucleus of the cell, and activates Tor, leading to growth. When there is a reduction in the IIS/TOR pathway activation (low-nutrition), FoxO enters in the nucleus of the cells, regulating thousands of genes, leading to lifespan extension. Continuous arrows represent activation of the pathway in an environment with high nutrition, for example, while dashed arrows represent the alternative pathway in environments with low nutrition. Genes within green, blue, or purple boxes represent those under intensified, purifying, and

relaxed selection in the current study, respectively. The box in the bottom right classifies the genes as pro- or anti-longevity based on their known effects on *Drosophila* lifespan (Tacutu et al. 2018). Figure made based on Kapahi and Zid 2004; Dutriaux et al. 2013; Texada et al. 2020; Denlinger 2022.

Table 3: Orthogroups under selection overlapping with the *Drosophila* aging genes list from GenAge database (Tacutu et al. 2018). Longevity influence classifies the orthologs as pro- or anti-longevity genes according to their effects on *Drosophila* lifespan. The asterisk (*) highlights orthogroups that are also part of the best evidence of positive selection (Table 1).

Flybase ID	Orthogroup	Gene Symbol	Gene name	Longevity Influence	Selection
FBgn0000257	OG0003218	*car*	carnation	pro	intensification
FBgn0014141	OG0000645	*cher*	cheerio	pro	intensification
FBgn0024248	OG0003925	*chico*	Insulin receptor substrate-1	anti	intensification
FBgn0030092	OG0001411	*fh*	frataxin homolog	pro	intensification
FBgn0000659	OG0002632	*fkh*	fork head	pro	intensification
FBgn0002566	OG0004524	*lt*	light	pro	intensification
FBgn0040305	OG0003808	*MTF-1*	Metal response element-binding Transcription Factor-1	pro	intensification
FBgn0015269	OG0005277	*Nf1*	Neurofibromin 1	pro	intensification
FBgn0037324	OG0001776	*Orco*	Odorant receptor co-receptor	anti	intensification
FBgn0037802	OG0003529	*Sirt6*	CG6284-PB	pro	intensification
FBgn0029117	OG0002841	*Surf1*	surfeit gene 1	anti	intensification
FBgn0000330	OG0005760	*cm*	carmine	pro	positive
FBgn0037574	OG0005827	*Coq2*	Coenzyme Q biosynthesis protein 2	anti	positive
FBgn0020309	OG0002667	*crol*	crooked legs	anti	positive
FBgn0023511	OG0001233	*Edem1*	CG3810 gene product from transcript CG3810-RC	anti	positive
FBgn0284245	OG0004982	*Ef1alpha48D*	Elongation factor 148D	pro	positive
FBgn0001087	OG0003271	*g*	garnet	pro	positive
FBgn0033238	OG0003667	*CG11165*	CG11165	pro	positive*
FBgn0260990	OG0005419	*yata*	CG1973 gene product from transcript CG1973-RA	pro	positive*
FBgn0010379	OG0005368	*Akt1*	CG4006 gene product from transcript CG4006-RA	pro	purifying
FBgn0031461	OG0004172	*daw*	dawdle	anti	purifying
FBgn0038197	OG0003188	*foxo*	Forkhead box, sub-group O	pro	purifying
FBgn0039044	OG0002793	*p53*	CG33336-PA	anti	purifying
FBgn0261549	OG0000874	*rdgA*	retinal degeneration A	anti	purifying
FBgn0044452	OG0005320	*Atg2*	Autophagy-specific gene 2	pro	relaxation
FBgn0034366	OG0003358	*Atg7*	Autophagy-specific gene 7	pro	relaxation
FBgn0035169	OG0001599	*CG13890*	CG13890	pro	relaxation
FBgn0030512	OG0004420	*CG9940*	CG9940	pro	relaxation
FBgn0262656	OG0001307	*dm*	diminutive	anti	relaxation
FBgn0000490	OG0004605	*dpp*	decapentaplegic	pro	relaxation
FBgn0026479	OG0002567	*Drp1*	Dynamin related protein 1	pro	relaxation
FBgn0000629	OG0002208	*E(z)*	Enhancer of zeste	anti	relaxation
FBgn0287768	OG0002603	*esg*	escargot	anti	relaxation
FBgn0004507	OG0005350	*GlyP*	Glycogen phosphorylase	anti	relaxation
FBgn0038074	OG0004077	*Gnmt*	Glycine N-methyltransferase	pro	relaxation

FBgn0028427	OG0002615	*Ilk*	Integrin linked kinase	anti	relaxation
FBgn0001257	OG0005397	*ImpL2*	Ecdysone-inducible gene L2	pro	relaxation
FBgn0283499	OG0001807	*InR*	Insulin-like 1 receptor	anti	relaxation
FBgn0028425	OG0003474	*JhI-21*	CG12317-PA	anti	relaxation
FBgn0010504	OG0003373	*kermit*	CG11546	pro	relaxation
FBgn0011455	OG0002494	*l(3)neo18*	l(3)neo18	anti	relaxation
FBgn0002719	OG0002827	*Men*	Malic enzyme	pro	relaxation
FBgn0014863	OG0003311	*Mlp84B*	Muscle LIM protein at 84B	pro	relaxation
FBgn0263316	OG0000776	*Mrp4*	Multidrug resistance protein 4 ortholog	pro	relaxation
FBgn0023000	OG0004888	*mth*	methuselah	anti/pro	relaxation
FBgn0051216	OG0003476	*Naam*	Naam gene product from transcript Naam-RA	pro	relaxation
FBgn0017566	OG0002132	*ND75*	NADH:ubiquinone reductase 75kD subunit precursor	anti	relaxation
FBgn0010222	OG0001520	*Nmdmc*	NAD-dependent methylenetetrahydrofolate dehydrogenase	pro	relaxation
FBgn0030844	OG0001081	*ppk23*	pickpocket 23	anti	relaxation
FBgn0030795	OG0001867	*ppk28*	pickpocket 28	anti	relaxation
FBgn0026379	OG0003488	*Pten*	CG5671 gene product from transcript CG5671-RB	pro	relaxation
FBgn0015805	OG0004681	*Rpd3*	CG7471-PA	anti	relaxation
FBgn0283472	OG0004887	*S6k*	RPS6-p70-protein kinase	anti	relaxation
FBgn0025391	OG0004306	*Scgdelta*	Sarcoglycan delta	pro	relaxation
FBgn0029783	OG0004551	*Sirt4*	Sirtuin 4	pro	relaxation
FBgn0029976	OG0003360	*snz*	snazarus (sorting nexin 17 azarus)	anti	relaxation
FBgn0037248	OG0001135	*Spargel*	Spargel	pro	relaxation
FBgn0039298	OG0005723	*to*	takeout	pro	relaxation
FBgn0040268	OG0001708	*Top3alpha*	Topoisomerase 3alpha	pro	relaxation
FBgn0021796	OG0004069	*Tor*	Target of rapamycin	anti	relaxation
FBgn0004397	OG0005595	*Vinc*	Vinculin	pro	relaxation

Regarding oxidative stress genes (Kramer et al. 2021), 22 out of 34 had orthologs among the bee orthogroups. Only the positive selection and the relaxation of selection groups included oxidative stress orthologs in their lists, although not exceeding the amount of overlap expected by chance (Hypergeometric test: $p > 0.7$).

Discussion

In this study, we tested the hypothesis that the evolutionary loss of prepupal diapause extends adult lifespan, a prerequisite for the evolution of sociality in bees (Batra 1966; Michener 1974; Carey 2001). Our findings revealed convergent signals of selection linked to the loss of prepupal diapause, with the majority of genes experiencing relaxed selection. There is no significant overlap between genes under selection and oxidative stress genes, indicating that reduction of damage caused by oxidative stress may not be an important mechanism for increasing adult

bee longevity, at least insofar as it is regulated by diapause. Nevertheless, there is a significant overlap between aging genes and genes under intensified selection, suggesting evolutionary processes are acting upon genes that could impact lifespan. Below we discuss specific pathways that may be relevant to the adult lifespan extension in the absence of prepupal diapause. These include endocrine pathways that regulate metamorphosis, the IIS/TOR lifespan regulator pathway, and genes related to cell cycle and DNA repair damage.

Loss of *PTTH/torso* promotes selection in the EGFR pathway

The loss of *PTTH and torso* along with positive selection in the *egfr15* and *SOS* genes (Figure 4) suggest a change in the dynamics of the metamorphosis pathways when prepupal diapause is lost. The onset of metamorphosis in holometabolous insects is regulated by the production and release of ecdysone by the prothoracic gland (PG) in the brain (Henrich et al. 1999). When PTTH binds to its tyrosine kinase receptor TORSO in the PG, the Ras/RAF/Erk pathway is activated, which stimulates the release of ecdysone (Pan and O'Connor 2021). Although unknown for bees, in other insects PTTH also plays a pivotal role in larval and pupal diapause. During diapause, the brain-PG axis is shut down, inhibiting PTTH production and release, subsequently reducing production of ecdysteroids, resulting in delayed metamorphosis (Fig. 4) (Denlinger 2022).

For a considerable period, PTTH/TORSO was considered the primary pathway for ecdysone production through the activation of the PG (Rewitz et al. 2009). However, there are at least four other tyrosine kinase receptors expressed in the PG. A recent study demonstrated that

most of these are dispensable, because EGFR is the only receptor whose absence prevents metamorphosis in flies (Cruz et al. 2020). This suggests EGFR is the ancestral ecdysone biosynthesis pathway, with PTTH/TORSO likely co-opted in holometabolous insects to finely adjust the timing of pupation in response to environmental cues (Shimell et al. 2018; Cruz et al. 2020). Recurrent *PTTH* and *torso* losses across species support the hypothesis that EGFR is the foundational pathway regulating metamorphosis (Cruz et al. 2020). Moreover, it suggests that PTTH/TORSO are dynamic elements susceptible to environmental influences (Skelly et al. 2019). Evidence of positive selection in two genes of the EGFR pathway indicates an amplified role of this pathway in the absence of *PTTH* and *torso* in bees (Figure 4).

The near-universal loss of *PTTH* and *torso* only in species lacking prepupal diapause suggests strong selection against this pathway in the species that go through diapause during development. In *Drosophila*, reducing expression of *PTTH* or *torso* delays pupariation(Rewitz et al. 2009; Shimell et al. 2018; Kang et al. 2023), while constitutively knocking down *PTTH* or *torso* in the PG results in prolonged adult lifespan, suggesting possible detrimental effects of *PTTH* and *torso* in adulthood (Kang et al. 2023). This aligns with the antagonist pleiotropy theory of aging (Williams 1957), where genes like *PTTH* and *torso* confer benefits early in life (e.g. promoting survival via diapause induction), but with detrimental effects later in life (reduction of adult lifespan), are favored by natural selection and contribute to aging.

In Hymenoptera, the loss of *PTTH* and *torso* appears linked to the evolution of sociality in bees, as all examined social bee genomes lack these genes (Costa et al. 2022) (Figure 1). Our findings

suggest that not only sociality, but also the loss of developmental diapause, predicts *PTTH* and *torso* loss, with the latter being a stronger predictor. Since the loss of developmental diapause is also a strong predictor of sociality in bees, future analysis of additional bee genomes will be necessary to fully separate these effects.

We propose that *PTTH* and *torso* loss is a consequence of the loss of prepupal diapause and precedes the evolution of sociality. Selection may act against the PTTH/TORSO pathway once it is no longer needed for diapause functions due to its lifespan reducing effects in adults. In this way, the adult lifespan extension requisite for eusociality may be a result of reduced antagonistic pleiotropy, freeing PTTH/TORSO from the selection shadow once their function in early life was eliminated.

Loss of developmental diapause relaxes selection in the IIS/TOR pathways

Several genes in the insulin/insulin-like signaling (IIS) and target of rapamycin (TOR) pathways are under relaxed selection in species that have lost prepupal diapause (Figure 5). These pathways are highly conserved among metazoans and are key regulators of aging (Guarente and Kenyon 2000; Bitto et al. 2015). IIS and TOR work in parallel and interactively, such that when either or both are downregulated, there is an increase in lifespan (Kapahi and Zid 2004; McCormick et al. 2011).

In our study, we find *Akt* and *FoxO*, which are pro-longevity genes in *Drosophila*, are under purifying selection in bee species that lost prepupal diapause, reinforcing the importance of

these genes, independent of the diapause stage. Most of the anti-longevity genes in this pathway are under relaxed selection (Figure 5). The exceptions are *pten* and *impL2*, which are under relaxed selection, but are pro-longevity genes in that they block the insulin pathway. Relaxed selection in genes of the IIS/TOR pathway has been associated with both reduction of lifespan, as observed in annual vs long-lived killifish (Cui et al. 2019), and increased lifespan as seen in long vs short-lived mammals (Liu et al. 2023). Moreover, aging genes are under relaxed selection in humans compared to chimpanzees, despite humans living approximately twice as long (Cui et al. 2019). Similarly, relaxed selection on genes in the IIS/TOR pathway in bees that have lost prepupal diapause could be associated with increased lifespan.

The hyperfunction theory of aging predicts that reduction in lifespan results from the inappropriate activation of IIS/TOR pathway when growth is no longer beneficial (Blagosklonny 2006; Gems 2022). In accordance with this hypothesis, relaxed selection in IIS/TOR genes could be associated with loss or shifts in function in some of the genes in this pathway, which could result in reduced activation and increased lifespan. However, IIS/TOR is an essential pathway for holometabolous development, so a complete loss of function is unlikely (Fernandez et al. 1995; Hansen et al. 2004; Dutriaux et al. 2013). Relaxed selection has also been associated with the evolution of new phenotypes through the release of constraint in traits that were under opposing selection pressures in different environments (Hunt et al. 2011). In Hymenoptera, relaxed selection of caste-biased genes preceded the evolution of sociality and could be the basis for phenotypic plasticity. Moreover, in honeybees, fast evolving genes were more likely to be differentially expressed between castes (Hunt et al. 2011).

Loss of constraint in the IIS/TOR pathway suggests that different responses to nutritional cues could evolve, a hallmark of caste differentiation in many social species (Kapheim 2017). Differences in diet, either qualitative or quantitative, are associated at some level with caste determination in all independent evolution of castes in bees (Richards and Packer 1994; Rehan and Richards 2010; Kapheim et al. 2011; Carnell et al. 2020; Starr 2020). A notable instance of a unique nutritional response is observed in honeybee queens. Consumption of royal jelly, a highly nutritious food, by the queens, leads to decreased activation of insulin pathway, resulting in lifespan extension and increased fertility (Corona et al 2007). This is the opposite effect of what is expected to other animals, in which a reduction of activity in the insulin pathway leads to reduced fertility (Ament et al. 2008). Relaxed selection on genes in the IIS/TOR pathway could allow for such reversals to evolve.

Interestingly, a link between nutrition, caste differentiation, and diapause has long been recognized (Hunt and Amdam 2005; Hunt 2006; Hunt et al. 2007). Our results suggest a possible mechanism for this connection. Specifically, shifts in the timing of diapause may have allowed molecular pathways important during development, such as IIS/TOR, to become co-opted for social evolution (Hunt and Amdam 2005).We suggest that relaxation of the IIS/TOR pathway, as consequence of the loss of prepupal diapause, precedes the evolution of sociality and could be related to lifespan extension and/or the evolution of new phenotypes in response to differences in nutrition.

Many cell cycle regulation and DNA damage repair genes are under positive selection in species lacking prepupal diapause

Many genes involved in cell cycle regulation and DNA damage repair are subject to selection in bee species that have lost prepupal diapause. These two biological processes are crucial for diapause and aging mechanisms.

Diapause in the adult stage may require specific adaptations to maintain cell cycle arrest without accumulating damage, given the lack of somatic tissue renewal in adults of holometabolous insects (Tatar and Yin 2001). For instance, Fizzy (*fzr*), which is under positive selection in bee species lacking prepupal diapause, is involved in the natural response to injuries in adult flies. Endocycles (consecutive cycles of cell growth and arrest in the M phase), rather than mitosis, is the default response to injury in adult flies (Cohen et al. 2018). However, the expression of *fzr* alone is sufficient to override the endocycle response, activating mitosis after injury instead (Cohen et al. 2018). Positive selection of the *fzr* gene in bees that have lost prepupal diapause suggests a potential mechanism to prevent organ injuries under adverse environmental conditions, which could indirectly extend adult lifespan.

Other genes involved in cell cycle regulation and cell proliferation control, such as tumor suppressor genes, are under positive selection in species that have lost prepupal diapause. Examples include *G1/S-specific cyclin-E1* and *cyclin-dependent kinase 14*, which regulates the transition between phases G1 and S of the cell cycle (Chen et al. 2019; Fagundes and Teixeira 2021), *ST7* and *TRIM33* that act as tumor suppressor genes (Charong et al. 2011; Xue et al.

2015), and *Kruppel-like factor 6,* a transcription factor involved in the progression of multiple malignant tumors (Sabatino et al. 2019). *p53*, which also regulates cell cycle arrest at both G1/S and G2/M checkpoints (Engeland 2018), is under purifying selection. However, it remains unclear how selection on these genes could promote lifespan extension.

DNA repair mechanisms are crucial in preventing aging. Genes like *INO80 complex subunit D*, which is involved in DNA recombination and DNA repair (Horigome et al. 2014; Poli et al. 2017), and *ASH2*, which acts upstream of cellular response to DNA damage stimulus (Burgess et al. 2014), are under positive selection and are potentially adaptive for lifespan extension. Additionally, gene ontology terms related to double-strand break repair and DNA recombination are enriched among the genes under relaxation of selection, suggesting loss of constraint and possible evolution of new phenotypes associated with DNA repair and DNA recombination in those bee species.

Conclusions

Our study supports the hypothesis that the loss of prepupal diapause is related to adult lifespan extension. Our findings indicate that this extension is not primarily attributable to managing oxidative stress damage. Instead, it appears to stem from a combination of factors, such as the loss of *PTTH* and *torso*, coupled with a relaxation of selection in the IIS/TOR pathway. This relaxation of selection, due to the loss of prepupal diapause, may facilitate the emergence of new phenotypes, such as increased lifespan or altered responses to nutritional signals, two important adaptations in the evolution of castes in social insects. Given the strong correlation

between loss of prepupal diapause and the evolution of social behavior in bees, disentangling the effects of each phenotype on genome evolution proves challenging. Therefore, advancing our understanding of the link between diapause and the evolution of sociality demands expanding our understanding of the natural history of species presenting different life history traits. Solitary bee species lacking prepupal diapause offer ideal opportunities for investigating lifespan extension, examining signs of relaxed selection in *PTTH* and *torso* (if these genes are still present) compared to solitary species retaining prepupal diapause, and exploring potential innovations in how IIS/TOR pathway responds to diverse stimuli.

Material and Methods

Genomes

For the comparative genomics analysis, we obtained bee genomes from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/datasets), the National Genomics Data Center-China National Center for Bioinformation (NGDC-CNCB) (https://ngdc.cncb.ac.cn/), and the Halictid Genome Browser (https://beenomes.princeton.edu). Specifically, we selected genomes for which annotation files were accessible and for which we possessed information regarding the diapause stage and the social level of the bees.

With the genome and annotation files, we used the agat-v0.9.2 software (Dainat 2023) to retrieve the longest isoform for each gene. Subsequently, based on the longest isoform, we extracted and translated the nucleotide coding sequences (CDS) for each gene. The protein

coding sequences were then used to run BUSCO-v5.3.2 (Simao et al. 2015) against the Hymenoptera database, which encompasses 5991 genes. To ensure data quality, we retained genomes in which > 80% of the proteins were classified as complete by BUSCO.

In the genome selection process, we retained one genome per genus, opting for the genome with the highest percentage of complete proteins compared to the BUSCO database. More than one genome per genus was kept in cases where distinct life history traits, such as solitary versus social versus parasitic lifestyle, or variations in the diapause stage (prepupa versus adult) were evident. Ultimately, a total of 27 genomes were included in the subsequent analysis (Figure 1).

Identification of Orthogroups

The protein coding sequences from the 27 different species served as input for Orthofinder-v2.5.4 (Emms and Kelly 2015) to classify orthogroups. To proceed with further analysis, single-copy orthologs were necessary. Orthofinder identified 2592 orthogroups with single-copy orthologues. To augment the number of orthogroups for subsequent analysis, we filtered the "Orthogroups.GeneCounts" table (output from Orthofinder) to retain orthogroups present in all species, but single-copy in at least 70% of those species. This process yielded 4173 orthogroups. In instances where species had paralogs, all copies were excluded from the orthogroups before conducting the molecular evolution analysis, resulting in some orthogroups containing fewer than 27 species.

Orthogroups nucleotide alignments and trees

We compiled the nucleotide coding sequences of all species into a single file to construct a database. Utilizing the IDs of each sequence belonging to an orthogroup (generated using the protein sequences), we retrieved the nucleotide sequences by comparing them with the database. Species with more than one orthologous sequence (paralogs) in a given orthogroup were excluded. Subsequently, we employed the orthogroup nucleotide sequences as input for the MACSE_ALFIX_v01.sif pipeline, which encompasses codon alignment, and pre- and post-filtering (Ranwez et al. 2021,https://hal.science/hal-03099847/document). The orthogroup OG0003883 was excluded from the subsequent analysis due to its alignment never concluding.

The resulting nucleotide alignment underwent filtering by removing positions with excessive ambiguous data using the pxclsq function from the phyx-v1.3 software (Brown et al. 2017). Specifically, alignment columns missing information for over 50% of the species were eliminated. The filtered alignment was then used as input for iqtree2-v2.2.0 (Kalyaanamoorthy et al. 2017; Minh et al. 2020) to generate phylogenetic trees for each orthogroup with the options -st CODON and -m MFP, allowing for the automatic identification of the best codon model for each alignment.

Molecular evolution analysis

RERconverge-v0.3.0 (Kowalczyk et al. 2019) was used to compute convergent evolutionary rate shifts associated with the loss of developmental diapause. The input comprised nucleotide alignments from MACSE and the species tree generated in Orthofinder. Some branches in the species tree were adjusted using Mesquite-v3.81 (Maddison and Maddison 2023) to align with

the topology presented in (Almeida et al. 2023). The `estimatePhangornTreeAll` function was employed to predict the orthogroups tree while maintaining the same topology as the species tree. Seventeen species that had lost prepupal diapause (Figure 1) were used as foreground branches. Correlations were computed using the terminal clades with unidirectional transitions. A permulation analysis was conducted to calculate empirical p-values, as outlined in https://rdrr.io/github/nclark-lab/RERconverge/f/vignettes/PermulationWalkthrough.Rmd. Orthogroups exhibiting significant shifts in evolutionary rates were identified based on corrected permulation p-values < 0.05. Orthogroups with Rho negative (Rho-) values indicate a slower evolutionary rate in the foreground species, possibly reflecting increased selective constraint. Conversely, Rho positive (Rho+) values denote genes undergoing faster evolution, which could result from a release of selective constraint or adaptation. For additional details on the RERconverge pipeline, refer to the GitHub repository.

To ascertain whether orthogroups exhibiting Rho+ values indicate an accelerated evolutionary rate due to positive selection or relaxation of selection, we ran Hyphy-v2.5.55 (Kosakovsky Pond et al. 2020) aBSREL (Smith et al. 2015) and RELAX (Wertheim et al. 2015). aBSREL identifies signals of positive selection in the tested branches, whereas RELAX discerns signals of intensification selection (K > 1, representing positive or purifying selection) and relaxation of selection (K < 1). For both aBSREL and RELAX analyses, we used the nucleotide alignment from MACSE and the corresponding trees from IQ-TREE as input files. Test branches were specified as species that have lost developmental diapause. The parameters --srv (yes) and --multiple-hits

(Double+Triple) were employed to allow site-to-site synonymous rate variation and multiple simultaneous hits.

Gene ontology enrichment

To identify gene ontology enrichment in orthogroups associated with the loss of developmental diapause, we categorized the orthogroups of interest into four groups: possible positive selection, possible purifying selection, possible relaxation of selection, and possible intensification of selection. Possible positive selection included significant orthogroups with Rho+ values in RERconverge and significant orthogroups from AbsREL that did not overlap with relaxation of selection. Possible purifying selection included significant orthogroups with Rho- values from RERconverge that did not overlap with aBSREL or relaxation of selection. Orthogroups possibly under relaxation of selection were those significant in RELAX (K<1) that did not overlap with aBSREL or RERconverge (Rho+ and Rho-) (Figure 2A). Lastly, orthogroups under intensification of selection were those significant in RELAX (K>1) that did not overlap with aBSREL or RERconverge (Rho+ and Rho-) (Figure 2B). The latter was considered a separate category because it is not possible to differentiate between positive and purifying selection.

Gene ontology (GO) terms were annotated for the 4173 orthogroups using InterProScan-v5.63 (Jones et al. 2014) and the databases CDD, Pfam, SUPERFAMILY, and PANTHER. Out of these, 3267 orthogroups had at least one GO term annotated, forming the gene universe for the enrichment analysis. We used topGO-v2.50.0 (Alexa and Rahnenfuhrer 2023) with Fisher's exact

527 test to identify enriched terms in each one of the previously described categories. Terms were
528 considered significant if they had a p-value < 0.1 and Annotated genes > 1.
529
530 **Gene family evolution**
531 To assess changes in gene families associated with the loss of developmental diapause, we
532 employed CAFE-v5.0 (Mendes et al. 2020) following the pipeline outlined in
533 https://github.com/harish0201/Analyses_Pipelines/blob/main/7.CAFE.sh. Our input files
534 comprised the Orthogroup.GeneCount.tsv table and the species tree generated in Orthofinder.
535 The same tree employed in RERconverge (with adjusted branches) was used, except that we
536 transformed it into an ultrametric tree using the make_ultrametric.py script from Orthofinder.
537
538 Then, cafe5 was executed for gene families (orthogroups) containing a maximum of 100 genes
539 in a particular species. We analyzed the Base_count.tab output file to identify orthogroups that
540 were gained or lost based on the diapause stage (prepupae diapause or loss of developmental
541 diapause). Filtering was performed for all species collectively and for specific bee families with
542 more than one species in each category (Apidae and Halictidae) (Table S4) using a custom bash
543 script.
544
545 **PGLS**
546 We conducted a Phylogenetic Generalized Least Squares (PGLS) analysis, using the R packages
547 ape-v5.7.1 (Paradis and Schliep 2019) and caper-v1.0.3 (Orme et al. 2023), to test whether the
548 diapause stage and sociality are significant predictors for the presence or absence of the

549 prothoracicotropic hormone (*PTTH*) gene and its receptor tyrosine-protein kinase receptor
550 torso (*torso*). The dataset for this analysis included the 27 species used in the molecular
551 evolution analysis (Figure 1), along with *Exoneura robusta* and *Exoneurella tridentata*. The
552 phylogeny, generated by Orthofinder, encompasses all species, and some branches were
553 adjusted using Mesquite-v3.81 to align with the topology presented in (Almeida et al. 2023).
554 Additionally, a table of traits, including diapause stage, sociality, and presence/absence of
555 PTTH/torso, was utilized (Table S6).
556
557 Verification of the presence of *PTTH* was verified in the orthogroups file and a Blast search of
558 the *Megachile rotundata* protein coding sequence copy against the genome and protein
559 databases of each species (when available). On the other hand, the presence of *torso* was
560 verified solely in the orthogroup file. As all species that have lost *PTTH* also lost *torso*, we
561 treated them as a single trait (Table S6). To model these traits, we used function pgls from the R
562 package caper-v1.0.3. Because diapause stage and sociality are correlated variables (Fisher's
563 exact test; p=0.0001), we employed the model with each variable one at a time (PTTH.TORSO ~
564 Prepupae_Diapause; and PTTH.TORSO ~ Sociality) or both together (PTTH.TORSO ~
565 Prepupae_Diapause + Sociality). The presence/absence of *PTTH* and *torso* was set as a binary
566 trait, with 0 indicating the absence and 1 indicating the presence of the orthogroups. Diapause
567 stage and sociality were set as categorical variables, yes/no for the presence or absence or
568 prepupal diapause, and solitary/social for social status.
569
570 **Aging/longevity and oxidative genes**

We compared the genes under selection with genes previously associated with aging/longevity or oxidative stress in other species. For aging-related genes, we used the list of the *Drosophila* aging-related genes obtained from the GenAge database (accessed in January 2024: https://genomics.senescence.info/genes/index.html, (Tacutu et al. 2018)). We utilized Entrez IDs to retrieve equivalent gene IDs and CDS nucleotide sequences from FlyBase (https://flybase.org, Gramates et al. 2022). Concerning oxidative stress genes, we used as reference the list of genes present in at least one of four Hymenoptera species from (Kramer et al. 2021) and downloaded the sequences from Flybase based on the gene name.

For both sets of genes, we performed a reciprocal blast search against the 4172 orthogroup sequences combined in one file. Initially, a tblastn was conducted using either the aging or oxidative stress genes as the database and the orthogroups sequenced as query. Subsequently, using blastx, we used all orthogroups sequences as the database and either the aging or oxidative stress genes as queries. The reciprocal best hits were identified using the searchRBH.sh script adapted from https://morphoscape.wordpress.com/2020/08/18/reciprocal-best-hits-blast-rbhb/. In the final table, we included both orthogroups IDs and gene IDs based on the sequence IDs from the fasta files using a custom script.

To assess whether any of our gene groups under selection (positive, purifying, relaxation, or intensification) were enriched for aging or oxidative stress genes, we used the phyper function from the hypeR-v2.0.0 R package (Federico and Monti 2020).

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Data availability

All scripts used in the analyses are available at:

https://github.com/kapheimlab/Comparative_genomics_loss_prepupal_diapause

References

Alexa A, Rahnenfuhrer J. 2023. topGO: Enrichment Analysis for Gene Ontology. R package version 2.50.0.

Almeida EAB, Bossert S, Danforth BN, Porto DS, Freitas F V., Davis CC, Murray EA, Blaimer BB, Spasojevic T, Ströher PR, et al. 2023. The evolutionary history of bees in time and space. *Current Biology* 33:3409-3422.e6.

Ament SA, Corona M, Pollock HS, Robinson GE. 2008. Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. 105.

Batra SWT. 1966. Social behavior and nests of some Nomiine beed in India (Hymenoptera, Halictidae). *Insectes Soc* 8:145–154.

Bitto A, Wang AM, Bennett CF, Kaeberlein M. 2015. Biochemical genetic pathways that modulate aging in multiple species. *Cold Spring Harb Perspect Med* 5.

Blagosklonny M V. 2006. Aging and immortality: Quasi-programmed senescence and its pharmacologic inhibition. *Cell Cycle* 5:2087–2102.

Brown JW, Walker JF, Smith SA. 2017. Phyx: Phylogenetic tools for unix. *Bioinformatics* 33:1886–1888.

Burgess RC, Burman B, Kruhlak MJ, Misteli T. 2014. Activation of DNA Damage Response Signaling by Condensed Chromatin. *Cell Rep* 9:1703–1717.

Carey JR. 2001. Demographic mechanisms for the evolution of long life in social insects. *Exp Gerontol* [Internet] 36:713–722. Available from: www.elsevier.nl/locate/expgero

Carnell JD, Hulse RA, Hughes WOH. 2020. A review of nutrition in bumblebees: The effect of caste, life-stage and life history traits. In: Advances in Insect Physiology. Vol. 59. Academic Press Inc. p. 71–129.

Charong N, Patmasiriwat P, Zenklusen JC. 2011. Localization and characterization of ST7 in cancer. *J Cancer Res Clin Oncol* 137:89–97.

Chen L, Wang Y, Jiang W, Ni R, Wang Y, Ni S. 2019. CDK14 involvement in proliferation migration and invasion of esophageal cancer. *Ann Transl Med* 7.

Cohen E, Allen SR, Sawyer JK, Fox DT. 2018. Fizzy-Related dictates A cell cycle switch during organ repair and tissue growth responses in the Drosophila hindgut. *Elife* [Internet] 7:1–22. Available from: https://doi.org/10.7554/eLife.38327.001

Costa CP, Okamoto N, Orr M, Yamanaka N, Woodard SH. 2022. Convergent Loss of Prothoracicotropic Hormone , A Canonical Regulator of Development , in Social Bee Evolution. *Front Physiol* 13:1–6.

Cruz J, Martín D, Franch-Marro X. 2020. Egfr Signaling Is a Major Regulator of Ecdysone Biosynthesis in the Drosophila Prothoracic Gland. *Current Biology* 30:1547-1554.e4.

Cui R, Medeiros T, Willemsen D, Iasi LNM, Collier GE, Graef M, Reichard M, Valenzano DR. 2019. Relaxed Selection Limits Lifespan by Increasing Mutation Load. *Cell* 178:385-399.e20.

Dainat J. 2023. AGAT: Another Gff Analysis Toolkit to handle annotations in any GTF/GFF format. *https://www.doi.org/10.5281/zenodo.3552717*.

Denlinger DL. 2022. Insect Diapause. Cambridge University Press

Dutriaux A, Godart A, Brachet A, Silber J. 2013. The Insulin Receptor Is Required for the Development of the Drosophila Peripheral Nervous System. *PLoS One* 8:e71857.

Easwaran S, Montell DJ. 2023. The molecular mechanisms of diapause and diapause-like reversible arrest. *Biochem Soc Trans* 51:1847–1856.

Emms DM, Kelly S. 2015. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol* [Internet] 16:157. Available from: http://genomebiology.com/2015/16/1/157

Engeland K. 2018. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death Differ* 25:114–132.

Fagundes R, Teixeira LK. 2021. Cyclin E/CDK2: DNA Replication, Replication Stress and Genomic Instability. *Front Cell Dev Biol* 9.

Federico A, Monti S. 2020. hypeR: an R package for geneset enrichment workflows. *Bioinformatics* 36:1307–1308.

Fernandez R, Tabarini D, Azpiazu N, Frasch M, Schlessinger J. 1995. The Drosophila insulin receptor homolog: A gene essential for embryonic development encodes two receptor isoforms with different signaling potential. *EMBO Journal* 14:3373–3384.

Gauld I, Bolton B. 1988. The Hymenoptera. Oxford: Oxford University Press

Gems D. 2022. The hyperfunction theory: An emerging paradigm for the biology of aging. *Ageing Res Rev* 74.

Gilbert SF. 2000. The Hormonal Reactivation of Development. In: Developmental Biology. 6th ed. Sunderland, MA: Sinauer Associates.

Gramates LS, Agapite J, Attrill H, Calvi BR, Crosby MA, dos Santos Gilberto, Goodman JL, Goutte-Gattat D, Jenkins VK, Kaufman T, et al. 2022. FlyBase: a guided tour of highlighted features. *Genetics* 220.

Guarente L, Kenyon C. 2000. Genetic pathways that regulate ageing in model organisms. *Nature* [Internet] 408:255–262. Available from: www.nature.com

Hansen IA, Attardo GM, Park JH, Peng Q, Raikhel AS. 2004. Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. *Proc Natl Acad Sci U S A* 101:10626–10631.

Henrich VC, Rybczynski R, Gilbert LI. 1999. Peptide Hormones, Steroid Hormones, and Puffs: Mechanisms and Models in Insect Development. *Vitam Horm* 55:73–125.

Horigome C, Oma Y, Konishi T, Schmid R, Marcomini I, Hauer MH, Dion V, Harata M, Gasser SM. 2014. SWR1 and INO80 chromatin remodelers contribute to dna double-strand break perinuclear anchorage site choice. *Mol Cell* 55:626–639.

Hunt BG, Ometto L, Wurm Y, Shoemaker DW, Yi S V., Keller L, Goodisman MAD. 2011. Relaxed selection is a precursor to the evolution of phenotypic plasticity. *Proc Natl Acad Sci U S A* 108:15936–15941.

Hunt JH. 2006. Evolution of castes in *Polistes*. *Ann Zool Fennici* [Internet] 43:407–422. Available from: http://www.sekj.org/PDF/anzf43/anzf43-407.pdf

Hunt JH, Amdam GV. 2005. Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science (1979)* 308:264–267.

Hunt JH, Kensinger BJ, Kossuth JA, Henshaw MT, Norberg K, Wolschin F, Amdam GV. 2007. A diapause pathway underlies the gyne phenotype in *Polistes* wasps, revealing an evolutionary route to caste-containing insect societies. *Proc Natl Acad Sci U S A* 104:14020–14025.

Hutfilz C. 2022. Endocrine Regulation of Lifespan in Insect Diapause. *Front Physiol* 13:1–18.

Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, et al. 2014. InterProScan 5: Genome-scale protein function classification. *Bioinformatics* 30:1236–1240.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14:587–589.

Kang P, Liu P, Kim J, Bolton M, Kumar A, Miao T, O MB, Powell-Coffman J, Bai H. 2023. Ptth regulates lifespan through innate immunity pathway in Drosophila. *bioRxiv* [Internet]. Available from: https://doi.org/10.1101/2023.09.30.560323

Kapahi P, Zid B. 2004. TOR pathway: linking nutrient sensing to life span. *Sci Aging Knowledge Environ* 2004.

Kapheim KM. 2017. Nutritional, endocrine, and social influences on reproductive physiology at the origins of social behavior. *Curr Opin Insect Sci* [Internet] 22:62–70. Available from: http://dx.doi.org/10.1016/j.cois.2017.05.018

Kapheim KM, Bernal SP, Smith AR, Nonacs P, Wcislo WT. 2011. Support for maternal manipulation of developmental nutrition in a facultatively eusocial bee, Megalopta genalis (Halictidae). *Behav Ecol Sociobiol* 65:1179–1190.

Kosakovsky Pond SL, Poon AFY, Velazquez R, Weaver S, Hepler NL, Murrell B, Shank SD, Magalis BR, Bouvier D, Nekrutenko A, et al. 2020. HyPhy 2.5 - A Customizable Platform for Evolutionary Hypothesis Testing Using Phylogenies. *Mol Biol Evol* 37:295–299.

Kowalczyk A, Meyer WK, Partha R, Mao W, Clark NL, Chikina M. 2019. RERconverge: An R package for associating evolutionary rates with convergent traits. *Bioinformatics* 35:4815–4817.

Kramer BH, Nehring V, Buttstedt A, Heinze J, Korb J, Libbrecht R, Meusemann K, Paxton RJ, Séguret A, Schaub F, et al. 2021. Oxidative stress and senescence in social insects: A significant but inconsistent link? *Philosophical Transactions of the Royal Society B: Biological Sciences* 376.

Liu W, Zhu P, Li Meng, Li Z, Yu Y, Liu G, Du J, Wang X, Yang J, Tian R, et al. 2023. Large-scale across species transcriptomic analysis identifies genetic selection signatures associated with longevity in mammals. *EMBO J* 42.

Maddison WP, Maddison DR. 2023. Mesquite: a modular system for evolutionary analysis. Version 3.81. *http://www.mesquiteproject.org* [Internet]. Available from: http://www.mesquiteproject.org

McCormick MA, Tsai SY, Kennedy BK. 2011. TOR and ageing: A complex pathway for a complex process. *Philosophical Transactions of the Royal Society B: Biological Sciences* 366:17–27.

Mendes FK, Vanderpool D, Fulton B, Hahn MW. 2020. CAFE 5 models variation in evolutionary rates among gene families. *Bioinformatics* 36:5516–5518.

Michener CD. 1969. Comparative Social Behavior of Bees. *Annu Rev Entomol* 14:299–342.

Michener CD. 1974. The social behavior of the bees : a comparative study. Cambridge: Harvard University Press

Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, Lanfear R, Teeling E. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol Biol Evol* 37:1530–1534.

O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, et al. 2016. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res* 44:D733–D745.

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2023. caper: Comparative Analyses of Phylogenetics and Evolution in R.

Pan X, O'Connor MB. 2021. Coordination among multiple receptor tyrosine kinase signals controls Drosophila developmental timing and body size. *Cell Rep* 36.

Paradis E, Schliep K. 2019. Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528.

Poli J, Gasser SM, Papamichos-Chronakis M. 2017. The INO80 remodeller in transcription, replication and repair. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372.

Ranwez V, Chantret N, Delsuc F. 2021. Aligning Protein-Coding Nucleotide Sequences with MACSE. In: p. 51–70.

Rehan SM, Richards MH. 2010. The influence of maternal quality on brood sex allocation in the small carpenter bee, Ceratina calcarata. *Ethology* 116:876–887.

Rewitz KF, Yamanaka N, Gilbert LI, O'Connor MB. 2009. The insect neuropeptide PTTH activates receptor tyrosine kinase torso to initiate metamorphosis. *Science (1979)* 326:1403–1405.

Richards MH, Packer L. 1994. Trophic aspects of caste determination in *Halictus ligatus*, a primitively eusocial sweat bee. *Behav Ecol Sociobiol* 34:385–391.

Sabatino ME, Castellaro A, Racca AC, Carbajosa González S, Pansa MF, Soria G, Bocco JL. 2019. Krüppel-Like Factor 6 Is Required for Oxidative and Oncogene-Induced Cellular Senescence. *Front Cell Dev Biol* 7.

Santos PKF, Arias MC, Kapheim KM. 2019. Loss of developmental diapause as a prerequisite for social evolution in bees. *Biol Lett* [Internet] 15:1–7. Available from: https://www.biorxiv.org/content/10.1101/649897v1#disqus_thread

Seifert AW, Monaghan JR, Smith MD, Pasch B, Stier AC, Michonneau F, Maden M. 2012. The influence of fundamental traits on mechanisms controlling appendage regeneration. *Biological Reviews* 87:330–345.

Shimell MJ, Pan X, Martin FA, Ghosh AC, Leopold P, O'Connor MB, Romero NM. 2018. Prothoracicotropic hormone modulates environmental adaptive plasticity through the control of developmental timing. *Development (Cambridge)* 145.

da Silva J. 2021. Life History and the Transitions to Eusociality in the Hymenoptera. *Front Ecol Evol* 9:1–20.

Simao FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* [Internet] 31:3210–3212. Available from: http://bioinformatics.oxfordjournals.org/cgi/doi/10.1093/bioinformatics/btv351

Skelly J, Pushparajan C, Duncan EJ, Dearden PK. 2019. Evolution of the Torso activation cassette, a pathway required for terminal patterning and moulting. *Insect Mol Biol* 28:392–408.

Smith MD, Wertheim JO, Weaver S, Murrell B, Scheffler K, Kosakovsky Pond SL. 2015. Less is more: An adaptive branch-site random effects model for efficient detection of episodic diversifying selection. *Mol Biol Evol* 32:1342–1353.

Starr CK. 2020. Encyclopedia of Social Insects. Springer International Publishing

Szathmáry E, Smith JM. 1995. The major evolutionary transitions. *Nature* 374:227–232.

Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, Diana E, Lehmann G, Toren D, Wang J, et al. 2018. Human Ageing Genomic Resources: new and updated databases. *Nucleic Acids Res* 46:D1083–D1090.

Tatar M, Yin C. 2001. Slow aging during insect reproductive diapause : why butterflies , grasshoppers and flies are like worms. *Exp Gerontol* 36:723–738.

Texada MJ, Koyama T, Rewitz K. 2020. Regulation of body size and growth control. *Genetics* 216:269–313.

Wertheim JO, Murrell B, Smith MD, Pond SLK, Scheffler K. 2015. RELAX: Detecting relaxed selection in a phylogenetic framework. *Mol Biol Evol* 32:820–832.

Williams GC. 1957. Pleiotropy, Natural Selection, and the Evolution of Senescence. *Evolution (N Y)* 11:398–411.

Wilson EO. 1971. The Insect Societies. Belknap Press of Harvard University Press

Xue J, Chen Y, Wu Y, Wang Z, Zhou A, Zhang S, Lin K, Aldape K, Majumder S, Lu Z, et al. 2015. Tumour suppressor TRIM33 targets nuclear β-catenin degradation. *Nat Commun* 6.