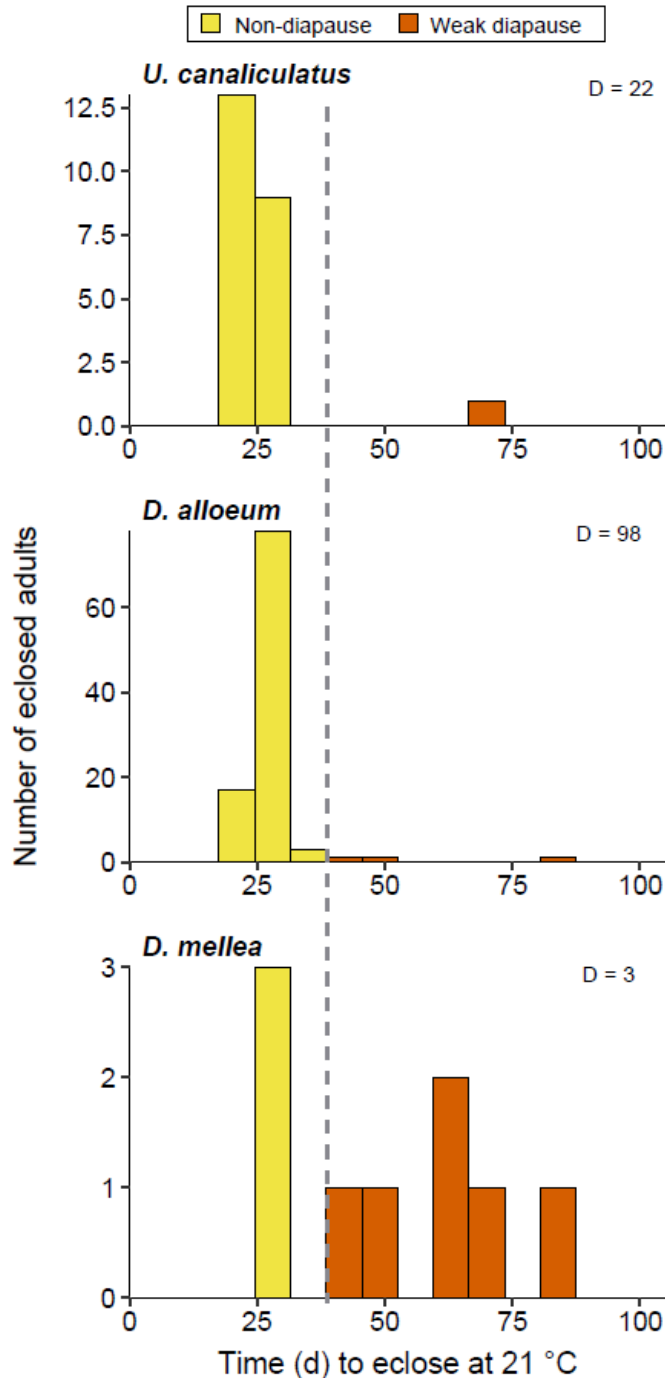


**Supplementary Figures and Tables for**  
**“Cold tolerance and diapause within across trophic levels: endoparasitic wasps and their**  
**fly host have similar phenotypes”**

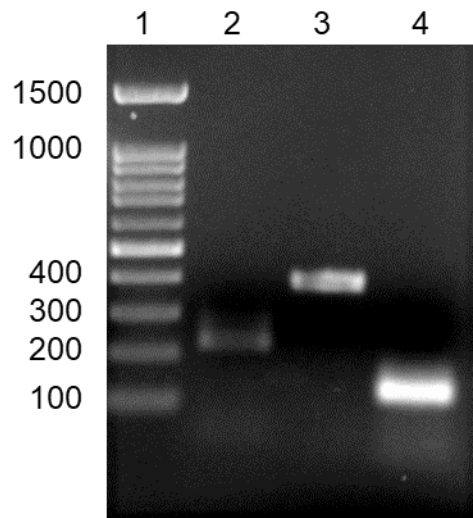
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**Figure S1.** Distribution of time to eclosion at 21°C for (A) *Utetes canaliculatus*, (B) *Diachasma alloenum*, and (C) *Diachasmimorpha mellea* in the absence of chilling. Each bar represents the total number of individuals for a particular species that eclosed in a 7-day period. Individuals to the left of the dashed line (early eclosing phenotype) were classified as non-diapause (ND); individuals to the right of the dashed line (late eclosing phenotype) were classified as weak diapause (WD). The number of wasps that remained unclosed after 100 days (prolonged diapause, D) is indicated in the top right of each panel.

**Table S1.** Comparison of the proportion  $\pm$  95% confidence interval and number (N) of pupae in each diapause class when diapause phenotype is determined via respirometry or eclosion time at 21°C. The 95% confidence intervals overlap for proportions calculated from either experiment (respirometry or eclosion time), therefore we pooled the data shown here for display in Fig. 1.

Species	Phenotyping experiment	Proportion in each diapause class (N)		
		Non-diapause	Weak diapause	Diapause
<i>Diachasma alloeum</i>	Respirometry	0.58 $\pm$ 0.16 (35)	0.03 $\pm$ 0.25 (2)	0.38 $\pm$ 0.20 (23)
	Eclosion time	0.79 $\pm$ 0.08 (98)	0.02 $\pm$ 0.17 (3)	0.19 $\pm$ 0.16 (23)
<i>Diachasmimorpha mellea</i>	Respirometry	0 (0)	0.03 $\pm$ 0.25 (2)	0.97 $\pm$ 0.05 (59)
	Eclosion time	0.03 $\pm$ 0.19 (3)	0.06 $\pm$ 0.19 (6)	0.91 $\pm$ 0.06 (91)
<i>Utetes canaliculatus</i>	Respirometry	0.86 $\pm$ 0.28 (6)	0 (0)	0.14 $\pm$ 0.69 (1)
	Eclosion time	0.96 $\pm$ 0.09 (22)	0.04 $\pm$ 0.4 (1)	0 (0)



**Figure S2.** Representative agarose gel of PCR products of a portion of the *cytochrome oxidase I (COI)* mitochondrial gene with the species-specific primers in Table 1. Each primer pair was used to amplify a product from DNA of one adult of the appropriate species (identified by morphology) using the PCR conditions described in the main text. Lanes: 1, ladder, with relevant sizes indicated to the left in bp (base pairs); 2, *Utetes canaliculatus*; 3, *Diachasma alloeum*; 4, *Diachasmimorpha mellea*.

EU881544.1	-----TGAGCTGGGGTG	12
D.alloenum	-----	0
KR890748.1	-----GTTTTGTATTTTTGTATGGGATTTGAGCTGGGGTG	36
KU511212.1	TCAACAAATCATAAAGATATTGGTGTTTGTATTTTTGTATGGGATTTGAGCTGGGGTG	60
EU881544.1	TTAGGTTTATCTATAAGAATAATTATTCGATTAGAATTAGGGATACCTGGGAGATTGTTA	72
D.alloenum	-----	0
KR890748.1	TTAGGTTTATCTATAAGAATAATTATTCGATTAGAATTAGGGATACCTGGGAGATTGTTA	96
KU511212.1	TTAGGTTTATCTATAAGAATAATTATTCGATTAGAATTAGGGATACCTGGGAGATTGTTA	120
EU881544.1	AATGATCAGATTTATAATAGAATAGTAACTGCTCATGCTTTTGTATAATTTTTTTTACA	132
D.alloenum	-----TTTGTATAAATTTTTTTTACA	21
KR890748.1	AATGATCAGATTTATAATAGAATAGTAACTGCTCATGCTTTTGTATAATTTTTTTTACA	156
KU511212.1	AATGATCAGATTTATAATAGAATAGTAACTGCTCATGCTTTTGTATAATTTTTTTTACA *****	180
EU881544.1	GTTATACCTATTATAAATGGGGGGTTGGGAATTGATTAATTCCTTTAATATTAGGGGTT	192
D.alloenum	GTTATACCTATTATAAATGGAGGGTTGGGAATTGATTAATTCCTTTAATATTAGGGGTT	81
KR890748.1	GTTATACCTATTATAAATGGAGGGTTGGGAATTGATTAATTCCTTTAATATTAGGGGTT	216
KU511212.1	GTTATACCTATTATAAATGGAGGGTTGGGAATTGATTAATTCCTTTAATATTAGGGGTT *****	240
EU881544.1	CCTGATATAGCCTTTCCTCGAATAAATAATATAAGATTTTGATTATTGAGACCTTCTATA	252
D.alloenum	CCTGATATAGCCTTTCCTCGAATAAATAATATAAGATTTTGATTATTGAGACCTTCTATA	141
KR890748.1	CCTGATATAGCCTTTCCTCGAATAAATAATATAAGATTTTGATTATTGAGACCTTCTATA	276
KU511212.1	CCTGATATAGCCTTTCCTCGAATAAATAATATAAGATTTTGATTATTGAGACCTTCTATA *****	300
EU881544.1	ATTTTGTTAATATTGAGAATATTATTAATTTAGGTGCTGGAACCTGGTTGGACAATTTAT	312
D.alloenum	ATTTTGTTAATATTGAGAATATTATTAATTTAGGTGCTGGAACCTGGTTGGACAATTTAT	201
KR890748.1	ATTTTGTTAATATTGAGAATATTATTAATTTAGGTGCTGGAACCTGGTTGGACAATTTAT	336
KU511212.1	ATTTTGTTAATATTGAGAATATTATTAATTTAGGTGCTGGAACCTGGTTGGACAATTTAT *****	360
EU881544.1	CCTCCTTTATCATCTAGATTAGGTCATAGGGGGTTAGCAGTAGATTTATTAATTTTTAGT	372
D.alloenum	CCTCCTTTATCATCTAGATTAGGTCATAGGGGGTTAGCAGTAGATTTATTAATTTTTAGT	261
KR890748.1	CCTCCTTTATCATCTAGATTAGGTCATAGGGGGTTAGCAGTAGATTTATTAATTTTTAGT	396
KU511212.1	CCTCCTTTATCATCTAGATTAGGTCATAGGGGGTTAGCAGTAGATTTATTAATTTTTAGT *****	420
EU881544.1	TTACATTTAGCTGGGGTATCATCAATTATGGGGGCAATTAATTTTATTTGTACAATTTTA	432
D.alloenum	TTACATTTAGCTGGGGTATCATCAATTATGGGGGCAATTAATTTTATTTGTACAATTTTA	321
KR890748.1	TTACATTTAGCTGGGGTATCATCAATTATGGGGGCAATTAATTTTATTTGTACAATTTTA	456
KU511212.1	TTACATTTAGCTGGGGTATCATCAATTATGGGGGCAATTAATTTTATTTGTACAATTTTA *****	480
EU881544.1	AATATAAAGCTTTTCATAAAGTTTGAGCAATTAAGTTTATTTATTTGGTCAATTTTAATT	492
D.alloenum	AATATAAAGCTTTTCATAAAGTTTGAGCAATTAAGTTTATTTATTTGGTCAATTTTAATT	355
KR890748.1	AATATAAAGCTTTTCATAAAGTTTGAGCAATTAAGTTTATTTATTTGGTCAATTTTAATT	516
KU511212.1	AATATAAAGCTTTTCATAAAGTTTGAGCAATTAAGTTTATTTATTTGGTCAATTTTAATT *****	540
EU881544.1	ACAGCTATTTTATTATTATTATCTTTACCGGTTTTAGCTGGAGCTATTACAATATTATTA	552
D.alloenum	-----	355
KR890748.1	ACAGCTATTTTATTATTATTATCTTTACCGGTTTTAGCTGGAGCTATTACAATA-----	570
KU511212.1	ACAGCTATTTTATTATTATTATCTTTACCGGTTTTAGCTGGAGCTATTACAATATTATTA	600

**Figure S3.** Alignment of the positive control *Diachasma alloenum* PCR amplicon to *cytochrome oxidase I (COI)* gene sequences from *D. alloenum* vouchers in NCBI. Default parameters were used in Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) for alignment Asterisks (\*) indicate exact matches between our sequence (*D. alloenum*) and the three NCBI sequences (identified by NCBI accession numbers).

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KT761492.1   TCAATAAGAATAAATTATTCGGATAGAATTAGGGGTCCTGGTAGGATATTGATAAGAGAT 60
KT761495.1   TCAATAAGAATAAATTATTCGGATAGAATTAGGGGTCCTGGTAGGATATTGATAAGAGAT 60
D.mellea     ----- 0
KT761488.1   TCAATAAGAATAAATTATTCGGATAGAATTAGGGGTCCTGGTAGGATATTGATAAGAGAT 60

KT761492.1   CAACTTTATAAATAGTATAGTTACTTCTCATGCTTTTGTATAAATTTTTTTTATAGTTATA 120
KT761495.1   CAACTTTATAAATAGTATAGTTACTTCTCATGCTTTTGTATAAATTTTTTTTATAGTTATA 120
D.mellea     -----TTACTTCTCATGCTTTTGTATAAATTTTTTTTATAGTTATA 41
KT761488.1   CAACTTTATAAATAGTATAGTTACTTCTCATGCTTTTGTATAAATTTTTTTTATAGTTATA 120
                *****

KT761492.1   CCTATTATAAATGGTGGGTTTGGTAATGGTGGTACCTTTAATATTAGGGGCTCCTGAT 180
KT761495.1   CCTATTATAAATGGTGGGTTTGGTAATGGTGGTACCTTTAATATTAGGGGCTCCTGAT 180
D.mellea     CCTATTATAAATGGTGGGTTTGGTAATGGTGGTACCTTTAATATTA----- 89
KT761488.1   CCTATTATAAATGGTGGGTTTGGTAATGGTGGTACCTTTAATATTAGGGGCTCCTGAT 180
                *****

KT761492.1   ATAGCTTCCCTCGAATAAATAATATAAGGTTTGGATTACTTGTTCCTTCTTTATTTTAA 240
KT761495.1   ATAGCTTCCCTCGAATAAATAATATAAGGTTTGGATTACTTGTTCCTTCTTTATTTTAA 240
D.mellea     ----- 89
KT761488.1   ATAGCTTCCCTCGAATAAATAATATAAGGTTTGGATTACTTGTTCCTTCTTTATTTTAA 240

KT761492.1   TTAATGTTGAGAAGATTATTAATTTGGGGGTTGGAAGTGGTGAACAGTTTATCCTCCA 300
KT761495.1   TTAATGTTGAGAAGATTATTAATTTGGGGGTTGGAAGTGGTGAACAGTTTATCCTCCA 300
D.mellea     ----- 89
KT761488.1   TTAATGTTGAGAAGATTATTAATTTGGGGGTTGGAAGTGGTGAACAGTTTATCCTCCA 300

KT761492.1   TTGTCATCAAATTTGGGGCATGTAGGTTTCATCCGTTGATTTAGCTATTTTTCTTTACAT 360
KT761495.1   TTGTCATCAAATTTGGGGCATGTAGGTTTCATCCGTTGATTTAGCTATTTTTCTTTACAT 360
D.mellea     ----- 89
KT761488.1   TTGTCATCAAATTTGGGGCATGTAGGTTTCATCCGTTGATTTAGCTATTTTTCTTTACAT 360

KT761492.1   TTGGCGGGAGTTTCTTCAATTATAGGGGCTATTAATTTTATTAGAACAATTTTGAATATA 420
KT761495.1   TTGGCGGGAGTTTCTTCAATTATAGGGGCTATTAATTTTATTAGAACAATTTTGAATATA 420
D.mellea     ----- 89
KT761488.1   TTGGCGGGAGTTTCTTCAATTATAGGGGCTATTAATTTTATTAGAACAATTTTGAATATA 420

KT761492.1   AATTTTTATATAAATTAATTAGATCAGTTAAGTTTATTAATTTGGTCAATTTAATTACG 480
KT761495.1   AATTTTTATATAAATTAATTAGATCAGTTAAGTTTATTAATTTGGTCAATTTAATTACG 480
D.mellea     ----- 89
KT761488.1   AATTTTTATATAAATTAATTAGATCAGTTAAGTTTATTAATTTGGTCAATTTAATTACG 480

KT761492.1   GCTATTTTATTGTTGTTATCTTTGCCTGTTTTAGCTGGTGCATTACTATGTTGTTAACT 540
KT761495.1   GCTATTTTATTGTTGTTATCTTTGCCTGTTTTAGCTGGTGCATTACTATGTTGTTAACT 540
D.mellea     ----- 89
KT761488.1   GCTATTTTATTGTTGTTATCTTTGCCTGTTTTAGCTGGTGCATTACTATGTTGTTAACT 540

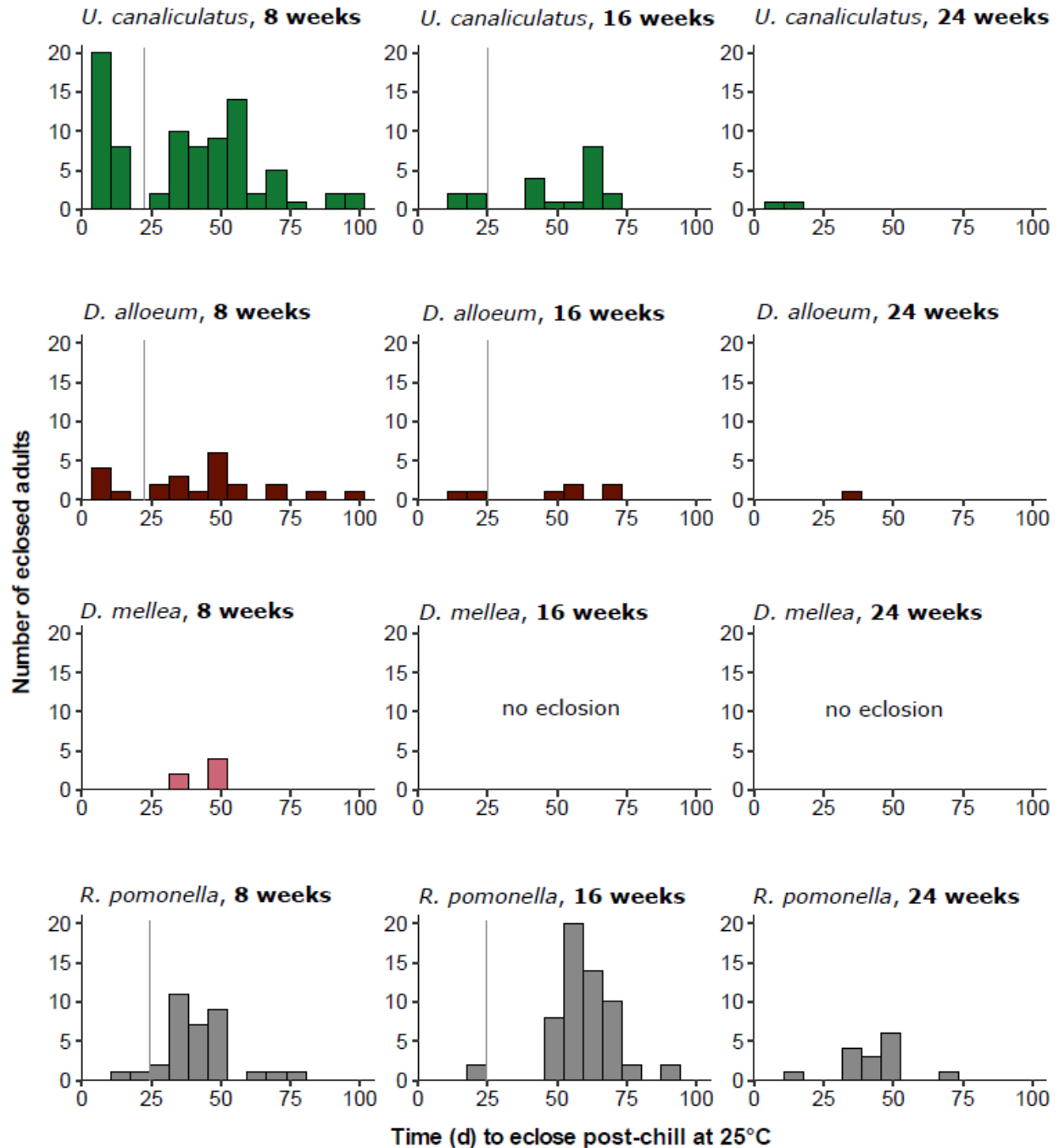
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D.mellea     ----- 89
KT761488.1   GATCGAAATTTAAATACG      558

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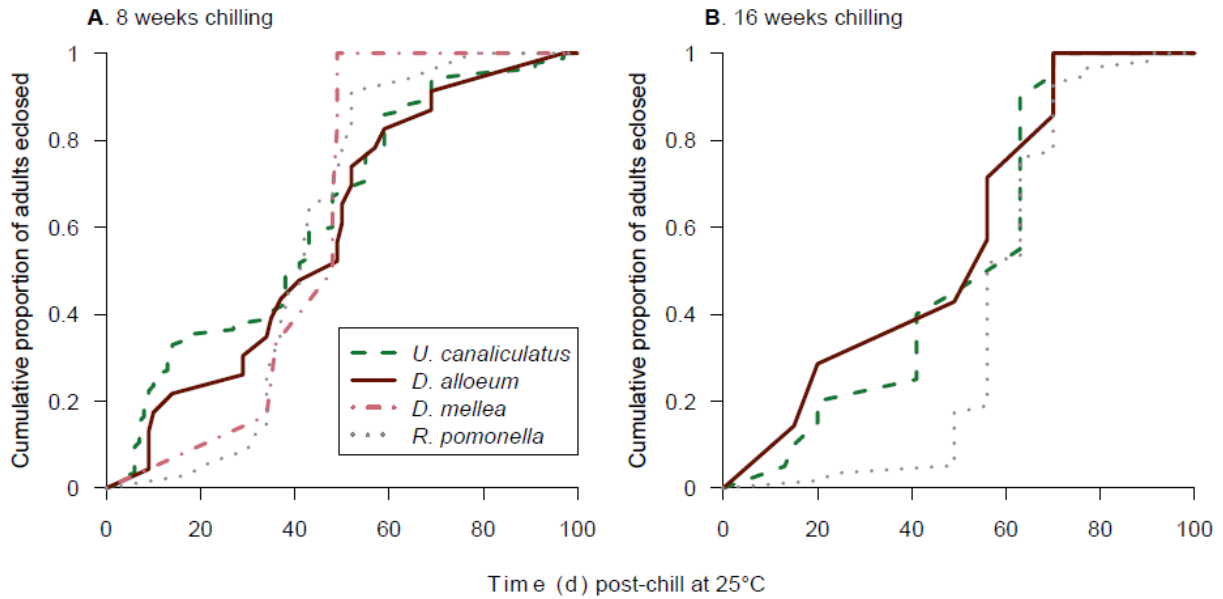
**Figure S4.** Alignment of the positive control *Diachasmimorpha mellea* PCR amplicon to *cytochrome oxidase I (COI)* gene sequences from *D. mellea* vouchers in NCBI. Default parameters were used in Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) for alignment Asterisks (\*) indicate exact matches between our sequence (*D. mellea*) and the three NCBI sequences (identified by NCBI accession numbers).

KU511220.1	TCTGGTATAGTAGGTTTATCAATAAGATTAATTATTTCGTATAGAATTAGGTGTTCCCTGGA	60
KT761328.1	-----CCTGGA	6
U. canaliculatus	-----	0
KT761344.1	-----CCTGGA	6
KU511220.1	AGATTATTAATAAATGATCAAATTTATAATAGAATAGTTACGGCACATGCTTTTGTAAATA	120
KT761328.1	AGATTATTAATAAATGATCAAATTTATAATAGAATAGTTACGGCACATGCTTTTGTAAATA	66
U. canaliculatus	-----	0
KT761344.1	AGATTATTAATAAATGATCAAATTTATAATAGAATAGTTACGGCACATGCTTTTGTAAATA	66
KU511220.1	ATTTTTTTTATAGTTATGCCAATTATAAATGGTGGTTTTGGTAATTGATTAATTCCTTTA	180
KT761328.1	ATTTTTTTTATAGTTATGCCAATTATAAATGGTGGTTTTGGTAATTGATTAATTCCTTTA	126
U. canaliculatus	-----	0
KT761344.1	ATTTTTTTTATAGTTATGCCAATTATAAATGGTGGTTTTGGTAATTGATTAATTCCTTTA	126
KU511220.1	ATATTAGGAGCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGTTTTTGATTATTA	240
KT761328.1	ATATTAGGAGCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGTTTTTGATTATTA	186
U. canaliculatus	-----	0
KT761344.1	ATATTAGGAGCTCCTGATATAGCTTTCCCTCGTATAAATAATATAAGTTTTTGATTATTA	186
KU511220.1	ATTCCTTCATTAATATTATTAATTTTGAGAAGATTATTAATGTTGGTGTGGTACTGGT	300
KT761328.1	ATTCCTTCATTAATATTATTAATTTTGAGAAGATTATTAATGTTGGTGTGGTACTGGT	246
U. canaliculatus	-----Tgttggtggtggtactggt	19
KT761344.1	ATTCCTTCATTAATATTATTAATTTTGAGAAGATTATTAATGTTGGTGTGGTACTGGT	246
	*****	
KU511220.1	TGAACAGTTTATCCACCTTTATCTTCAACATTAGGTCATGGTGGATTATCTGTTGATTTA	360
KT761328.1	TGAACAGTTTATCCACCTTTATCTTCAACATTAGGTCATGGTGGGTATCTGTTGATTTA	306
U. canaliculatus	tgaacagtttatccacctttatcttcaacattaggtcatgggtgggttatctggtgattta	79
KT761344.1	TGAACAGTTTATCCACCTTTATCTTCAACATTAGGTCATGGTGGGTATCTGTTGATTTA	306
	*****	
KU511220.1	GCTATTTTTTCTTTACATTTAGCAGGTGTTTCTTCAATTATAGGGGCTATTAATTTTATT	420
KT761328.1	GCTATTTTTTCTTTACATTTAGCAGGTGTTTCTTCAATTATAGGGGCTATTAATTTTATT	366
U. canaliculatus	gctatTTTTTCTTTACATTTAGCAGGTGTTTCTTCAATTATAGGGGCTATTAATTTTATT	139
KT761344.1	GCTATTTTTTCTTTACATTTAGCAGGTGTTTCTTCAATTATAGGGGCTATTAATTTTATT	366
	*****	
KU511220.1	ACTACTATTTTTAATATAAAATTTTTTTATAATTAAGTTAGATCAATTAAGATTATTAATT	480
KT761328.1	ACTACTATTTTTAATATAAAATTTTTTTATAATTAAGTTAGATCAATTAAGATTATTAATT	426
U. canaliculatus	actactatTTTTAATATAAAATTTTTTTATAATTAAGTTAGATCAATTAAGATTATTAATT	186
KT761344.1	ACTACTATTTTTAATATAAAATTTTTTTATAATTAAGTTAGATCAATTAAGATTATTAATT	426
	*****	
KU511220.1	TGATCAATTTTAATTACAGCTATTTTATTATTATTATCTTTACCAGTATTAGCTGGAGCT	540
KT761328.1	TGATCAATTTTAATTACAGCTATTTTATTATTATTATCTTTACCAGTATTAGCTGGAGCT	486
U. canaliculatus	-----	186
KT761344.1	TGATCAATTTTAATTACAGCTATTTTATTATTATTATCTTTACCAGTATTAGCTGGAGCT	486
KU511220.1	ATTACGATATTATTAAGTATCGAAATTTAAATACATCATTTTTTGATTTTTCTGGTGGG	600
KT761328.1	ATTACGATATTATTAAGTATCGAAATTTAAATACATCATTTTTTGATTTTTCTGGT---	543
U. canaliculatus	-----	186
KT761344.1	ATTACTATATTATTAAGTATCGAAATTTAAATACATCATTTTTTGATTTTTCTGGT---	543

**Figure S5.** Alignment of the positive control *Utetes canaliculatus* PCR amplicon to *cytochrome oxidase I (COI)* gene sequences from *U. canaliculatus* vouchers in NCBI. Default parameters were used in Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) for alignment Asterisks (\*) indicate exact matches between our sequence (*U. canaliculatus*) and the three NCBI sequences (identified by NCBI accession numbers).



**Figure S6.** Distribution of eclosion phenology for *Rhagoletis pomonella* and the parasitoids that attack the fly (*Diachasma alloenum*, *Diachasmimorpha mellea*, *Utetes canaliculatus*) at 25°C after 8, 16, or 24 weeks of chilling at 4°C. For each chill treatment (column), 325 pupae (parasitized and unparasitized) were chilled. Each bar represents the total number of individuals for a particular species that eclosed in a 7-day period (1 week). Individuals with eclosion times less than (to the left of) the vertical grey line likely never entered diapause (non-diapause).



**Figure S7.** Cumulative number of adult flies and parasitoid species that eclosed within 100 days at 25°C after chilling at 4°C for (A) 8 weeks or (B) 16 weeks. These data are the same as in Fig. S6, but expressed as proportions rather than total numbers of eclosed individuals. Pairwise differences in eclosion times were assessed via Kolmogorov-Smirnov (K-S) tests. The K-S tests revealed that *U. canaliculatus* and *R. pomonella* had different eclosion distributions from each other after 8 weeks ( $D = 0.329$ ,  $P = 0.011$ ) and 16 weeks chilling ( $D = 0.366$ ,  $P = 0.038$ ). No other pairwise comparisons were significant ( $P > 0.05$ ). Insufficient wasps eclosed after 24 weeks chilling ( $< 2$  per wasp species) to plot proportion eclosion. However, all data including the 24 week treatment are displayed in Fig. S6.