SUPPLEMENTARY MATERIAL

Figures

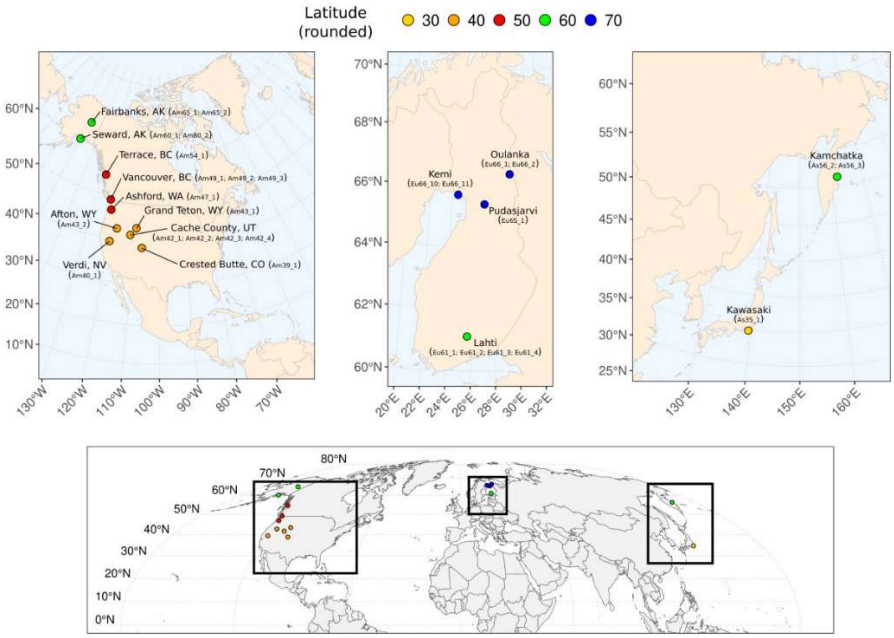

Figure S1. Sampling locations of the strains used in this study.

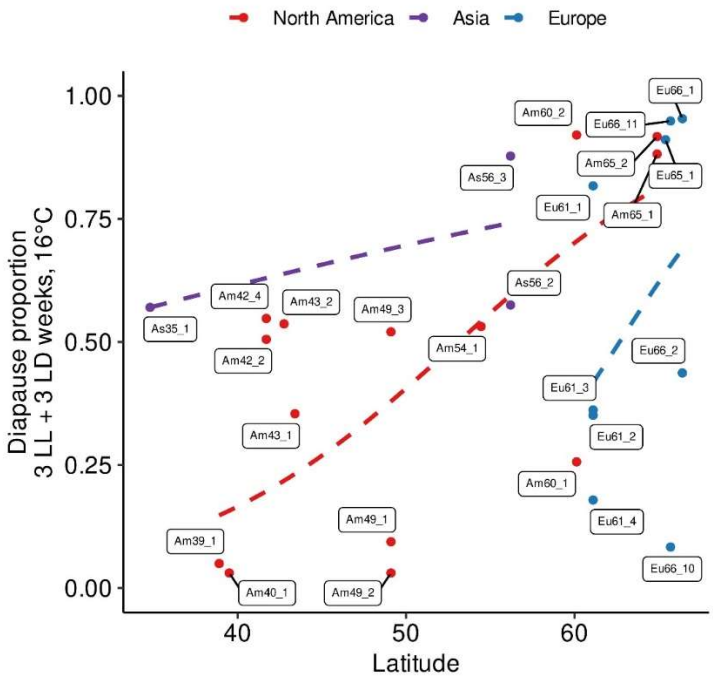

Figure S2. Correlation between the proportion of females that had entered post-reproductive diapause proportion when maintained 3 weeks in LL and 3 weeks in LD 12:12 (Y axis) and the latitude of origin (X-axis) for *D. montana* strains from different continents. A total of 2979 females (average = 115) were used in these experiments across 26 lines.

Tables

Table S1. Sample sizes for the photoperiodic response curves (PPRCs) used to estimate the CDLs of the study strains in 4-11 photoperiods in 16ºC. For most strains, the CDLs were already estimated in Lankinen et al. (2021). In the present study we estimated the CDLs of 6 new strains (Am65_2, Am60_1, Am60_2, Am54_1, Am43_2 and Am42_3; marked with *) and added new data on a few photoperiods for some strains.

Strain	Number of photoperiods	Total sample size
Eu66_1	8	1 883
Eu66_2	8	1 608
Eu66_10	5	1 588
Eu66_11	9	1 984
Eu65_1	8	982
Eu61_1	9	2 308
Eu61_2	8	1 194
Eu61_3	8	1 559
Eu61_4	7	1 202
Am65_1	12	3 247
Am65_2*	5	599
Am60_1*	6	838
Am60_2*	5	811
Am54_1*	5	701
Am49_1	8	2 909
Am49_2	7	1 208
Am49_3	11	3 917
Am47_1	4	974
Am43_1	9	1 833
Am43_2*	8	1 316
Am42_1	7	1 357
Am42_2	8	1 120
Am42_3*	7	1 201
Am42_4	8	1 345
Am40_1	7	1 396
Am39_1	7	2 177
As56_2	10	2 079
As56_3	9	2 860
As35_1	7	1424

Table S2. The first three columns show strain code, the length of experiment (21 or 42 days) and the number of days that the females were maintained in continuous light (LL) before they were transferred in LD 12:12. The next columns show the number (N) of females with developed and non-developed ovaries and the total number of the studied females (T). The last column shows the number of vials where the females of given strains had been divided.

Strain	Length of experiment	Number of days in LL	Females with developed ovaries (N)	Females with non-developed ovaries (N)	Total number of females (T)	Number of vials
Eu66_1	21 days	1	0	16	16	1
Eu66_1	21 days	2	0	32	32	2
Eu66_1	21 days	3	0	50	50	4
Eu66_1	21 days	4	0	35	35	2
Eu66_1	21 days	5	0	27	27	2
Eu66_1	21 days	6	0	27	27	2
Eu66_1	21 days	7	0	21	21	2
Eu66_1	21 days	8	2	39	41	3
Eu66_1	21 days	11	2	12	14	1
Eu66_1	21 days	13	3	29	32	3
Eu66_1	42 days	1	0	44	44	4
Eu66_1	42 days	2	0	35	35	3
Eu66_1	42 days	3	0	41	41	3
Eu66_1	42 days	4	0	40	40	3
Eu66_1	42 days	5	0	35	35	4
Eu66_1	42 days	6	0	31	31	3
Eu66_1	42 days	7	0	38	38	4
Eu66_1	42 days	8	0	23	23	2
Eu66_1	42 days	9	0	36	36	4
Eu66_1	42 days	10	0	25	25	2
Eu66_1	42 days	11	0	35	35	4
Eu66_1	42 days	12	0	39	39	3
Eu66_1	42 days	13	0	25	25	2
Eu61_1	21 days	1	0	32	32	3
Eu61_1	21 days	2	0	68	68	5
Eu61_1	21 days	3	0	30	30	3
Eu61_1	21 days	4	0	57	57	4
Eu61_1	21 days	5	3	46	49	3
Eu61_1	21 days	6	2	44	46	3
Eu61_1	21 days	7	10	67	77	6
Eu61_1	21 days	8	29	74	103	8
Eu61_1	21 days	9	41	110	151	14
Eu61_1	21 days	10	49	97	146	13
Eu61_1	21 days	11	48	64	112	7
Eu61_1	21 days	12	89	72	161	10
Eu61_1	21 days	13	55	48	103	6
Eu61_1	42 days	1	2	15	17	3
Eu61_1	42 days	2	2	30	32	3
Eu61_1	42 days	3	0	18	18	2
Eu61_1	42 days	4	2	36	38	4
Eu61_1	42 days	5	3	20	23	3
Eu61_1	42 days	6	2	30	32	3
Eu61_1	42 days	7	2	39	41	2

Eu61_1	42 days	8	1	23	24	2
Eu61_1	42 days	9	3	20	23	3
Eu61_1	42 days	10	2	31	33	3
Eu61_1	42 days	11	0	26	26	3
Eu61_1	42 days	12	1	32	33	2
Eu61_1	42 days	13	2	29	31	2
Eu61_2	21 days	1	0	24	24	3
Eu61_2	21 days	2	0	26	26	4
Eu61_2	21 days	3	2	28	30	4
Eu61_2	21 days	4	8	27	35	4
Eu61_2	21 days	5	9	16	25	4
Eu61_2	21 days	6	17	33	50	5
Eu61_2	21 days	7	17	26	43	5
Eu61_2	21 days	8	47	25	72	6
Eu61_2	21 days	9	32	9	41	4
Eu61_2	21 days	10	39	19	58	5
Eu61_2	21 days	11	37	9	46	5
Eu61_2	21 days	12	25	14	39	4
Eu61_2	21 days	13	27	3	30	3
Eu61_2	42 days	1	0	17	17	2
Eu61_2	42 days	2	0	25	25	2
Eu61_2	42 days	3	0	28	28	2
Eu61_2	42 days	4	1	15	16	2
Eu61_2	42 days	5	0	16	16	2
Eu61_2	42 days	6	2	26	28	2
Eu61_2	42 days	7	2	12	14	2
Eu61_2	42 days	8	1	18	19	2
Eu61_2	42 days	9	1	18	19	2
Eu61_2	42 days	10	3	27	30	2
Eu61_2	42 days	11	3	31	34	2
Eu61_2	42 days	12	1	36	37	2
Eu61_2	42 days	13	5	40	45	2

Table S3. The first three columns show the strain code, the temperature and the age of females in weeks. The next columns show the number of females with developed and non-developed ovaries (N), total number of females (T) and the ratio of the females with non-developed ovaries and the total number of females (N/T; proportion of females that had entered post-reproductive diapause). The last column shows the number of vials where the study females had been divided.

Strain	Tempe-rature ºC	Female age (weeks)	Females with developed ovaries	Females with non-developed ovaries (N)	Total number of females (T)	Ratio N/T	Number of vials
Eu66_1	16	3	312	41	353	0.12	24
Eu66_1	16	4	290	30	320	0.09	16
Eu66_1	16	5	78	118	196	0.60	14
Eu66_1	16	6	8	164	172	0.95	15
Eu66_2	16	3	97	1	98	0.01	6
Eu66_2	16	4	35	12	47	0.26	4
Eu66_2	16	5	67	34	101	0.34	7
Eu66_2	16	6	76	59	135	0.44	10
Eu66_2	16	9	34	121	155	0.78	13
Eu66_10	16	4	47	3	50	0.06	3
Eu66_10	16	5	40	7	47	0.15	4
Eu66_10	16	6	99	9	108	0.08	10
Eu66_10	16	9	61	22	83	0.27	7
Eu66_11	16	4	48	33	81	0.41	6
Eu66_11	16	5	23	42	65	0.65	7
Eu66_11	16	6	4	74	78	0.95	6
Eu65_1	16	4	77	14	91	0.15	9
Eu65_1	16	5	62	40	102	0.39	12
Eu65_1	16	6	9	92	101	0.91	10
Eu65_1	16	9	2	60	62	0.97	8
Eu61_1	16	4	110	6	116	0.05	10
Eu61_1	16	5	78	28	106	0.26	10
Eu61_1	16	6	15	67	82	0.82	8
Eu61_1	16	9	1	44	45	1.00	4
Eu61_2	16	4	46	16	62	0.26	5
Eu61_2	16	5	59	7	66	0.11	6
Eu61_2	16	6	74	40	114	0.35	14
Eu61_2	16	9	17	108	125	0.86	12
Eu61_3	16	4	58	0	58	0	4
Eu61_3	16	5	27	0	27	0	2
Eu61_3	16	6	30	17	47	0.36	4
Eu61_3	16	9	7	41	48	0.85	4
Eu61_3	16	12	4	37	41	0.90	NA
Eu61_4	16	4	28	1	29	0.03	4
Eu61_4	16	5	99	3	102	0.03	8
Eu61_4	16	6	78	17	95	0.18	11
Eu61_4	16	9	7	122	129	0.95	10
Am65_1	16	4	27	10	37	0.27	4
Am65_1	16	5	32	116	148	0.78	13

Am65_1	16	6	27	201	228	0.88	22
Am65_1	16	9	2	55	57	0.96	6
Am65_2	16	4	57	23	80	0.29	8
Am65_2	16	5	30	68	98	0.69	10
Am65_2	16	6	14	155	169	0.92	17
Am65_2	16	9	1	104	105	0.99	10
Am60_1	16	4	35	0	35	0	5
Am60_1	16	5	84	2	86	0.02	12
Am60_1	16	6	87	30	117	0.26	18
Am60_1	16	9	25	17	42	0.40	5
Am60_2	16	3	27	4	31	0.13	2
Am60_2	16	6	9	104	113	0.92	9
Am60_2	16	9	3	36	39	0.92	6
Am54_1	16	5	16	9	25	0.36	3
Am54_1	16	6	30	34	64	0.53	7
Am54_1	16	9	8	78	86	0.90	11
Am49_1	16	3	90	0	90	0	7
Am49_1	16	6	106	11	117	0.09	13
Am49_1	16	9	37	25	62	0.40	10
Am49_2	16	3	64	0	64	0	6
Am49_2	16	5	28	1	29	0.03	2
Am49_2	16	6	95	3	98	0.03	9
Am49_2	16	9	50	40	90	0.44	9
Am49_3	16	4	48	6	54	0.11	5
Am49_3	16	5	71	19	90	0.21	8
Am49_3	16	6	47	51	98	0.52	11
Am49_3	16	9	0	30	30	1	5
Am43_1	16	4	132	7	139	0.05	14
Am43_1	16	5	121	15	136	0.12	13
Am43_1	16	6	124	68	192	0.35	23
Am43_1	16	9	8	136	144	0.94	14
Am43_2	16	5	19	5	24	0.21	2
Am43_2	16	6	19	22	41	0.54	4
Am43_2	16	9	5	28	33	0.85	4
Am42_2	16	4	28	4	32	0.13	3
Am42_2	16	5	49	0	49	0	4
Am42_2	16	6	47	48	95	0.51	8
Am42_2	16	9	11	98	109	0.90	8
Am42_4	16	4	17	0	17	0	2
Am42_4	16	5	47	4	51	0.08	5
Am42_4	16	6	19	23	42	0.55	6
Am42_4	16	9	4	47	51	0.92	8
Am40_1	16	3	29	1	30	0.03	2
Am40_1	16	5	29	1	30	0.03	2
Am40_1	16	6	95	3	98	0.03	9
Am40_1	16	9	83	21	104	0.20	12
Am39_1	16	3	75	1	76	0.01	7

Am39_1	16	6	191	10	201	0.05	16
Am39_1	16	9	76	88	164	0.54	15
As56_2	16	4	87	11	98	0.11	9
As56_2	16	5	90	25	115	0.22	13
As56_2	16	6	51	69	120	0.58	14
As56_2	16	9	25	102	127	0.80	13
As56_3	16	4	66	42	108	0.39	7
As56_3	16	5	41	86	127	0.70	11
As56_3	16	6	18	129	147	0.88	11
As56_3	16	4	34	16	50	0.32	4
As35_1	16	5	23	14	37	0.38	4
As35_1	16	6	46	61	107	0.57	17
As35_1	16	9	1	114	115	0.99	11
Eu66_10	13	4	27	1	28	0.04	3
Eu66_10	13	5	37	1	38	0.03	4
Eu66_10	13	6	119	6	125	0.05	10
Eu66_10	13	7	150	28	178	0.16	15
Eu66_10	13	9	62	73	135	0.54	13
Eu66_10	13	12	11	88	99	0.89	NA
Eu61_1	13	5	23	14	37	0.38	3
Eu61_1	13	6	11	35	46	0.76	3
Eu61_1	13	7	11	40	51	0.78	5
Eu61_1	13	9	1	31	32	0.97	2
Eu61_2	13	5	65	1	66	0.02	7
Eu61_2	13	6	61	8	69	0.12	6
Eu61_2	13	7	56	42	98	0.43	10
Eu61_2	13	9	3	62	65	0.95	8
Eu61_3	13	5	58	7	65	0.11	5
Eu61_3	13	6	20	33	53	0.62	6
Eu61_3	13	7	46	61	107	0.57	8
Eu61_3	13	9	4	76	80	0.95	7
Eu61_4	13	4	16	0	16	0	1
Eu61_4	13	5	11	3	14	0.21	2
Eu61_4	13	6	11	37	48	0.77	3
Eu61_4	13	7	14	55	69	0.80	8
Eu61_4	13	9	0	47	47	1	6
Am49_1	13	7	48	10	58	0.17	5
Am49_1	13	9	38	43	81	0.53	8
Am49_1	13	12	23	38	61	0.62	10
Am49_2	13	7	82	7	89	0.08	8
Am49_2	13	9	56	32	88	0.36	9
Am49_2	13	12	40	81	121	0.67	12
Am49_3	13	5	30	3	33	0.09	3
Am49_3	13	6	42	12	54	0.22	5
Am49_3	13	7	18	41	59	0.69	6
Am49_3	13	9	3	75	78	0.96	10
Am47_1	13	4	37	0	37	0	2

Am47_1	13	5	64	2	66	0.03	5
Am47_1	13	6	51	4	55	0.07	6
Am47_1	13	7	62	2	64	0.03	6
Am47_1	13	9	108	17	125	0.14	13
Am47_1	13	12	71	16	87	0.18	8
Am43_1	13	4	25	0	25	0	3
Am43_1	13	5	88	3	91	0.03	7
Am43_1	13	6	75	32	107	0.30	10
Am43_1	13	7	42	94	136	0.69	10
Am43_1	13	9	10	80	90	0.89	9
Am42_1	13	5	50	0	50	0	4
Am42_1	13	6	64	1	65	0.02	6
Am42_1	13	7	89	4	93	0.04	11
Am42_1	13	9	87	27	114	0.24	15
Am42_1	13	12	30	47	77	0.61	10
Am42_2	13	6	17	3	20	0.15	3
Am42_2	13	7	7	3	10	0.3	2
Am42_2	13	9	3	16	19	0.84	3
Am42_3	13	7	42	2	44	0.05	7
Am42_3	13	9	50	36	86	0.42	11
Am42_3	13	12	16	29	45	0.64	6
Am42_4	13	6	22	5	27	0.19	3
Am42_4	13	7	20	19	39	0.49	5
Am42_4	13	9	7	69	76	0.91	8
Am40_1	13	5	29	0	29	0	2
Am40_1	13	6	31	0	31	0	5
Am40_1	13	7	124	3	127	0.02	13
Am40_1	13	9	93	44	137	0.32	19
Am40_1	13	12	18	37	55	0.67	7
Am39_1	13	4	25	0	25	0	2
Am39_1	13	5	43	3	46	0.07	4
Am39_1	13	6	87	10	97	0.10	8
Am39_1	13	7	103	14	117	0.12	10
Am39_1	13	9	43	81	124	0.65	10
Am39_1	13	12	14	46	60	0.77	7
As56_2	13	4	50	5	55	0.09	5
As56_2	13	5	75	12	87	0.14	7
As56_2	13	6	42	35	77	0.45	6
As56_2	13	7	50	86	136	0.63	11
As56_2	13	9	16	96	112	0.86	9
As35_1	13	5	52	20	72	0.28	5
As35_1	13	6	16	10	26	0.38	4
As35_1	13	7	12	53	65	0.82	7
As35_1	13	9	4	27	31	0.87	5

Table S4. Summary of the Chi² test used to identify strains with a secondary diapause. Strain code and the experimental temperature (ºC) are followed by the age of females (weeks) used in the Chi² test at the start and at the end of the experiment. Next columns show the number of females with developed ovaries (N) and the total number of females (T) at the start and at the end of the diapause inducing LD. p-value was obtained from the chi² test for each strain on 2 by 2 contingency table with the number of females that had developed ovaries and total number of females at the start and end of experiment.

Strain	Tempe-rature ºC	Female age (weeks) at start	Female age (weeks) at end	Females with dev ovaries at start (N)	Total number of females at start (T)	Females with dev ovaries at end (N)	Total number of females at end (T)	p-value
Eu66_1	16	3	6	312	353	8	172	0,00000
Eu66_2	16	3	9	97	98	34	155	0,00000
Eu66_10	16	4	9	47	50	61	83	0,42335
Eu66_11	16	4	6	48	81	4	78	0,00000
Eu65_1	16	4	9	77	91	2	62	0,00000
Eu61_1	16	4	9	110	116	1	45	0,00000
Eu61_2	16	4	9	46	62	17	125	0,00000
Eu61_3	16	4	12	58	58	4	41	0,00000
Eu61_4	16	4	9	28	29	7	129	0,00000
Am65_1	16	4	9	27	37	2	57	0,00000
Am65_2	16	4	9	57	80	1	105	0,00000
Am60_1	16	4	9	35	35	25	42	0,18554
Am60_2	16	3	9	27	31	3	39	0,00006
Am54_1	16	5	9	16	25	8	86	0,00006
Am49_1	16	3	9	90	90	37	62	0,05734
Am49_2	16	3	9	64	64	50	90	0,02515
Am49_3	16	4	9	48	54	0	30	0,00001
Am43_1	16	4	9	132	139	71	87	0,00000
Am43_2	16	5	9	19	24	5	33	0,00498
Am42_2	16	4	9	28	32	11	109	0,00000
Am42_4	16	4	9	17	17	4	51	0,00001
Am40_1	16	3	9	29	30	83	104	0,62328
Am39_1	16	3	9	75	76	76	164	0,00055
As56_2	16	4	9	87	98	25	127	0,00000
As56_3	16	4	6	66	108	18	147	0,00000
As35_1	16	4	9	34	50	1	115	0,00000
Eu66_10	13	4	12	27	28	11	99	0,00000
Eu61_1	13	5	9	23	37	1	32	0,00051
Eu61_2	13	5	9	65	66	3	65	0,00000
Eu61_3	13	5	9	58	65	4	80	0,00000
Eu61_4	13	4	9	16	16	0	47	0,00000
Am49_1	13	7	12	48	58	23	61	0,017
Am49_2	13	7	12	82	89	40	121	0,00002
Am49_3	13	5	9	30	33	3	78	0,00000
Am47_1	13	4	12	37	37	71	87	0,56231

Am43_1	13	4	9	25	25	10	90	0,00000
Am42_1	13	5	12	50	50	30	77	0,00194
Am42_2	13	6	9	17	20	3	19	0,02439
Am42_3	13	7	12	42	44	16	45	0,00954
Am42_4	13	6	9	22	27	7	76	0,00000
Am40_1	13	5	12	29	29	18	55	0,00480
Am39_1	13	4	12	25	25	14	60	0,00054
As56_2	13	4	9	50	55	16	112	0,00000
As35_1	13	5	9	52	72	4	31	0,00171

Table S5. Summary of the results of the analysis of deviance of the models compared in this study. For each model, we used a binomial model (link function "logit") with the diapause proportion at post-reproductive diapause as the response variable and the continent of origin of the strains, CDL and latitude as explanatory variables. We tested each variable individually and compared the effects on the fit of the model of adding each variable successively. We report here the residual deviance, degree of freedom (df) and the AIC (Akaike's information criterion) of each model, and the estimated coefficient of each variable used in the model. All values reported here are significant (p value < 0.05).

			Continent of origin			**CDL**	**Latitude**
Residual Deviance	**df**	**AIC**	Europe	North America	Asia		
1538.81*	25						
1452.87	23	1573.5	-0.5	-0.99	0.81		
677.38	24	796.04				0.57	
1142.7	24	1261.4					0.08
506.24	23	629.9				2.40	-1.13
447.36	22	570.01	-2.17	-0.92	-11.78	0.75	
946.73	22	1069.4	-2.18	-1.31	-4.46		0.11
391.81	21	516.47	-1.72	-0.76	-13.03	1.03	-0.07

* Null Deviance