

Figure S1: Apns genes and gene regions based on nanopore reads. Related to Figures 2A&B. The two densovirus genes are shown in the middle of each figure, each represented by a grey box. Nearby genes are shown to the right or left of the *Apns* genes also shown with grey boxes, with their associated directionality, gene ID (ACYPI) number, and annotation shown. The bottom of each figure shows individual long nanopore reads, with nucleotides represented by different colors.

Apns-1: ACYPI085607

Α

В

Apns-2: ACYP36509



Figure S2: Additional expression and knock-down data. Related to Figures 2A & 2B: Apns gene expression from quantitative PCR broken down by genotype. Each plot shows the mean fold change in gene expression as measured by qRT-PCR in response to crowding. Each aphid genotype is shown along the x-axis, with the highly-inducible genotypes in orange to the left of each figure, and the weaklyinducible genotypes in blue to the right of the figures. Each biological replicate is shown with a point, with crowded treatment aphids in darker colors and solitary aphids in lighter colors, as indicated in the legends. The grey bars show the average differential expression of the gene in crowded versus solitary samples. C-H: Full results from the RNAi experiments. C & D: We performed two versions of the RNAi experiment. C: Injection-first method: we injected aphids with dsRNA, kept them at low density for 24 hrs, and then exposed them to high density for 24 hrs. D: Crowding-first method: we kept aphids at high density for 24 hrs and then injected them with dsRNA. E & F: Show the percent winged offspring born to aphids from the two experiments. The y-axes show the percent of winged offspring born to groups of 4 injected aphids on a single plant (each represented by a grey point). The light purple bars show the control (lacZ injected) treatment, the dark purple boxes show knock-down (Apns-1 injected) aphids, with the bars around the means representing standard error. Statistical significance (at p < r0.0167) is shown along the top of the figure with an asterix. Aphid genotypes are shown along the bottom of the figure. Note that that C is the same data is in Figure 2G in the main text. G & H: Show fecundity of aphids from the two experiments. The y-axes show the number of offspring (both winged and unwinged) born to groups of 4 injected aphids as above.

Figure S3

Α

Dysaphis	MVDVVVCDKVFNCSDIPDHIHDGSNSKNTGAQNGGRRYRSDTTTDTEEKTINVAEKTQLRSGVFVGRTTAEETKTTRIGE NIDRPDFFSDGHFGEFGRGRKRIKRDLSDARRETRRANAK
Apns-2	MFGSINGFYMQEEEGGENSYTGEEGQDDVDSVYQYSTDTNSECERGQSV
Apns-1	
Dysaphis Apns-2 Apns-1	ILETRYPKFRGLHEFFRSAVPTQQPGLCGMSAVDSISNSHVPFTPMEIDKECGKFRSVNVKSQALETDSAINPLAVASGIDNVFGKSGDGRERVVGMDEITTTDLSDNDAGYRKLARETF PRHSTFDGFQTEGGHSISHFSLDSFDISMAPGTFFHTDE-PKEMDDYEPGTRRAIGGMQHGGYSDEGENQGHVARGSPIYEHLLRRVVDRIRD
Dysaphis Apns-2 Apns-1	VRYLGSTARYVSDICVPQDREEFVQFIVDVRQLSGLSSQEQMRLVSCHDTHIHVVHTCAYSNSTCRCAWLSGSAIWRNRR VARHRRRVFAADISAIEWEAIYRYFTTNGHAIQDIESGGS IDFR-GNDRYISDVIVPRGPNGVDEVAKELQTMLRQFPPKLPVIVTAHGDHVHCVHVCRQSNSACRCIWLQRSVLYRQHG RRRLRRRVWAIRLTSSDWESIVRYFSTDGREPQQIDGVGS
Dysaphis	NARLCLRLKSLQEG-GYKIPGQESVVYKSVYESCRNIGREPPDKDQAGLDSERSAGGYQGEEKHEDGPGVRVWPKTLDELLVKYPCCPPEAFYNVKEFYNNPHLNHIDDNCKKIRV
Apns-2	YGGLRDGRNFIQEVRSRRTQGPEGNVEICVNEMPNDVPRE LGAFAEHQDGN-ANHSPVDEDIGYDEGGSNIKSVCVYPTSLKNLLYQYPCCPPDAFTNIKEFYLNSRLNTILEEDKKCKV
Apns-1	
Dysaphis	ALRNWCARIREWSVSEFNDYYSSGKITPYFNAYSRDKKQVYYDVQTSVQIAEELLCYQFDNDGDLISKFLYDV YAIVDKLVPKRNSMCVVSPPSAGKNFFFDAVASYFLNYGMFGTANKT
Apns-2	ALRNWCAIIRDWSINEFVEYYNKPNVKPYFNAYARGPGTVYYDIDKSVSIASELLMYQFENNDGLICRFLQ TLVNILDKKIPKRNSMLIHSPPSAGKNYFFDAVAAFFLNYGMFGTANKT
Apns-1	
Dysaphis	NNFSWADGAGKRLVLWNEPN YETFHVEKIKELLGGDTTRVHVKYKGDQPLQGPPIFLLTNN TLSICNDPAFADRLVTYEWKSAPFLKQYNKKLNPLFFYVLLTKWIKVVNPNNKS
Apns-2	NNFSFSDGAGKRLVINNEPNYEVYHLEKMKELLGGDTTRVHVKYKNDVPLQGPPIILLTNHYLSIINDPSFKDRLSVYTWISAPFLKMYNKKLSPLFFYHLLNKYNITYY
Apns-1	NNFSFSDGAGKRRFCLDSVSHWNILHIAFLQ



Myzus persicae (Green peach aphid) Myzus cerasi (Green peach aphid)

Dysaphis plantaginea (Black cherry aphid)

Acyrthosiphum pisum (Pea aphid)

Diuraphis noxia (Russian wheat aphid)

~65my

~45my

Figure S3: Additional alignment and phylogenetics data. Related to Figure 3: A: Protein alignment of the *Apns* genes and *Dysaphis plantaginea* densovirus. The alignment was made using T-Coffee [S1]. The conserved Parvovirus non-structural protein NSF superfamily domains are highlighted with bold font.
B: Expanded phylogenetics analysis with sequences from two additional aphid species genomes, *M. cerasi* (using annotated gene sequences) and *D. noxia* (using unannotated gene sequences). Some of these sequences may be viral (e.g., *M. cerasi* sequences 3 and 4), and not aphid genome integrations.
C: Species tree for the aphid species referred to in Figure S3B. The relationships among species are inferred from trees in Kim et al. [S2] and Hardy et al. [S3]. Divergence times are based on [S2].

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Phenotype	Treatment	(after QC)	Map Rate	to an exon	base pair	
Low	Solitary A	33,671,762	92.4%	24,260,414	34.0X	
Low	Solitary B	31,946,562	92.5%	24,153,236	33.5X	
Low	Solitary C	29,383,923	93.4%	23,422,910	32.5X	
Low	Solitary D	30,615,107	93.7%	24,522,911	34.1X	
Low	Crowded A	31,880,792	93.3%	25,096,752	34.9X	
Low	Crowded B	35,390,114	93.3%	29,238,732	40.6X	
Low	Crowded C	30,851,849	93.1%	29,238,732	40.6X	
Low	Crowded D	31,304,230	93.0%	24,397,186	33.9X	
High	Solitary A	33,541,281	92.4%	26,966,739	37.5X	
High	Solitary B	36,299,714	92.4%	29,540,256	41.0X	
High	Solitary C	32,230,382	92.9%	24,965,639	34.7X	
High	Solitary D	34,138,974	92.2%	25,912,185	36.0X	
High	Crowded A	34,255,944	91.9%	27,296,731	37.9X	
High	Crowded B	33,276,605	93.7%	27,586,767	38.3X	
High	Crowded C	35,795,297	93.0%	28,104,179	39.0X	
High	Crowded D	30,213,059	92.3%	22,958,488	31.9X	

Table S1. Mapping and read-calling statistics for the pooled RNAseq experiment. Related to "Pooled Gene Expression Study using RNAseq" in the STAR Methods. The 16 RNAseq libraries are listed to the left, with the number of reads sequenced after quality control, the percentage of reads that mapped to the aphid reference genome, the number of reads that could be uniquely assigned to an exon, and the coverage per base pair shown.

	Gene ID	Annotation	Highly-inducible genotypes		Weakly- inducible Genotypes		Ref. [S4]	
			Log₂ FC	FDR	Log₂ FC	FDR	Log₂ FC	Sig ?
1	ACYPI007418	Sodium/ potassium/ calcium exchanger 3	2.05	< 0.00001	1.64	< 0.00001	2.6	X
2	ACYPI066777	Uncharacterized (riboflavin aldehyde- forming enzyme)	2.07	< 0.00001	1.65	< 0.00001	1.7	x
3	ACYPI003450	Uncharacterized	1.25	< 0.001	0.82	0.0028		
4	ACYPI55757	Uncharacterized	2.49	0.061	2.21	< 0.001	1.7	x
5	ACYPI085607	Apns-1	1.14	< 0.001	0.51	> 0.99	1.7	X
6	ACYPI36509	Apns-2	1.25	0.020	0.72	> 0.99		
7	ACYPI000986	Glucose dehydrogenase	-0.94	0.049	0.06	> 0.99		
8	ACYPI087498	Uncharacterized	-1.87	0.058	0.09	> 0.99		
9	ACYPI063882	Uncharacterized	1.44	0.071	0.49	> 0.99		
10	ACYPI082575	Uncharacterized (thaumatin-like protein)	1.23	0.071	0.28	> 0.99	2.7	X
11	ACYPI32040	Uncharacterized	-2.92	0.071	0.01	> 0.99		
12	ACYPI006346	Uncharacterized	-0.67	0.071	0.06	> 0.99		
13	ACYPI000458	Uncharacterized	0.81	0.074	0.59	> 0.99		

Table S2. Differentially expressed genes in highly- and weakly-inducible pooled genotypes form RNAseq. Related to "Pooled Gene Expression Study using RNAseq" in the STAR Methods. The aphid genome gene ID (ACYPI) number (https://bipaa.genouest.org/is/aphidbase/) is given to the left of the figure along with the gene annotation. The log₂ Fold Change and False Discovery Rate (FDR) values are given for highly- and then weakly-inducible genotypes for each differentially expressed gene. At the right of the table, the log₂ Fold Change measured by a previous RNAseq study of a highly-inducible genotype is given, along with an X if the gene was significantly differentially expressed in response to crowding in that study.

Sequence name	Sequence ID and origin
Ambidensovirus	AWV66976.1 (Genbank)
Psyllid densovirus	YP_009256211.1 (Genbank)
Silkworm densovirus	NP_694834.1 (Genbank)
Mosquito densovirus	YP_002887625.1 (Genbank)
Cockroach densovirus	NP_051020.1 (Genbank)
Crayfish densovirus	YP_009134732.1 (Genbank)
Seastar densovirus	YP_009507340.1 (Genbank)
Myzus persicae densovirus	NP_874376.1 (Genbank)
Dysaphis plantaginea densovirus	ACG50803.1 (Genbank)
Bird cherry-oat aphid (<i>Rhopalosiphum padi</i>)	Rpa20011.t1 (Aphidbase)
Soybean aphid (Aphis glycines)	AG004882-RA (Aphidbase)
Green peach aphid (Myzus persicae)	MYZPE13164_0_v1.0_000125320.4 (Aphidbase)
Russian wheat aphid (Diuraphis noxia) – 1	JOTR01000921
Russian wheat aphid (Diuraphis noxia) – 2	JOTR01000437
Russian wheat aphid (<i>Diuraphis noxia</i>) – 3	JOTR010000290
Black cherry aphid (<i>Myzus cerasi</i>) – 1	Mca20013
Black cherry aphid (<i>Myzus cerasi</i>) – 2	Mca20257
Black cherry aphid (<i>Myzus cerasi</i>) – 3	Mca22591
Black cherry aphid (Myzus cerasi) – 4	Mca22592
Black cherry aphid (<i>Myzus cerasi</i>) – 5	Mca23530

Table S3. Densovirus genes and densovirus derived gene homologs. Related to Figure 3. Source of sequence information for the phylogenetics work, for both densoviral and viralinserted aphid genes. The sequence names that corresponds with Figures 3A, 3B, and S3B are listed to the left, and the ID number and source are listed to the right. SUPPLEMENTAL REFERENCES

- S1. Notredame, C., Higgins, D.G., and Heringa, J. (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. J. Mol. Biol. 302:205–17.
- S2. Kim, H., Lee, S., and Jang, Y. (2011). Macroevolutionary patterns in the Aphidini aphids (Hemiptera: Aphididae): diversification, host association, and biogeographic origins. PLoS One. 6:e24749.
- S3. Hardy, N.B., Peterson, D.A., and von Dohlen, C.D. (2015). The evolution of life cycle complexity in aphids: Ecological optimization or historical constraint? Evolution. 69:1423–32.
- S4. Vellichirammal, N.N., Madayiputhiya, N., and Brisson, J.A. (2016). The genomewide transcriptional response underlying the pea aphid wing polyphenism. Mol. Ecol. 25:4146–60.