



Polydnaviruses of Braconid Wasps Derive from an Ancestral Nudivirus

Annie Bézier, *et al.*

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conductor (Fig. 4D). These results may provide a spectroscopic basis for the field-induced DOS observed by specific heat (22) and nuclear magnetic resonance (23) measurements and are consistent with the Volovik effect (15), which predicts field-induced gapless excitations around the SC gap nodes in k space and outside the vortex core in real space.

The detection of a d -wave coherence factor of a high- T_c cuprate using vortices as controllable quasi-particle scattering centers establishes that vortices selectively activate those quasi-particle scattering channels that preserve the sign of the SC gap in k space. Moreover, our method provides a simple phase-sensitive probe of gap anisotropy that can be applied to other superconductors with other forms of anisotropic gap, such as p -wave and extended s -wave superconductors. Another variant on this method is to examine the coherence factors for scattering off conventional impurities at temperatures above T_c : This approach may provide a viable way to probe the nature of the order that develops in the pseudogap normal state (24, 25). Lastly, we note that Fourier-transform SI-STM is currently the only method to study the evolution of k -dependent electronic states as a function of

magnetic field and, in this respect, offers a useful tool for the study of a wide range of field-induced quantum phenomena (26).

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20. The presence of residual DOS at $B = 0$ indicates that a pair-breaking effect is not negligible in the present

sample, although the microscopic nature of pair-breaking remains to be determined. We postulate that the QPI peaks in $I_2(q, E, B)$ track the quasi-particle dispersion even in the presence of such pair-breaking. This assumption may be supported by the fact that B -induced change of quasi-particle dispersion determined by QPI is consistent with the increase of DOS in B .

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Materials and Methods
Figs. S1 and S2
Table S1
References

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Polydnviruses of Braconid Wasps Derive from an Ancestral Nudivirus

Annie Bézier,¹ Marc Annaheim,² Juline Herbinière,¹ Christoph Wetterwald,² Gabor Gyapay,⁴ Sylvie Bernard-Samain,⁴ Patrick Wincker,⁴ Isabel Roditi,² Manfred Heller,³ Maya Belghazi,⁵ Rita Pfister-Wilhem,² Georges Periquet,¹ Catherine Dupuy,¹ Elisabeth Huguet,¹ Anne-Nathalie Volkoff,⁶ Beatrice Lanzrein,² Jean-Michel Drezen^{1*}

Many species of parasitoid wasps inject polydnvirus particles in order to manipulate host defenses and development. Because the DNA packaged in these particles encodes almost no viral structural proteins, their relation to viruses has been debated. Characterization of complementary DNAs derived from braconid wasp ovaries identified genes encoding subunits of a viral RNA polymerase and structural components of polydnvirus particles related most closely to those of nudiviruses—a sister group of baculoviruses. The conservation of this viral machinery in different braconid wasp lineages sharing polydnviruses suggests that parasitoid wasps incorporated a nudivirus-related genome into their own genetic material. We found that the nudiviral genes themselves are no longer packaged but are actively transcribed and produce particles used to deliver genes essential for successful parasitism in lepidopteran hosts.

Comparative genomic studies have highlighted the role of symbiotic associations in evolution (1). Polydnviruses (PDVs) are virus-like particles associated with wasp species that parasitize lepidopteran larvae. PDV particles are injected along with the eggs of the wasp into the lepidopteran larvae (or eggs) and express proteins that interfere with host immune defenses, development, and physiology; this interference enables wasp larvae to survive and develop within the host (2). Viral particle production occurs exclusively in a specialized region of the wasp ovaries (the calyx), and the vertically transmitted virus does not initiate particle pro-

duction in the infected host tissues (3). The viral genome packaged in the particles is composed of multiple double-stranded DNA (dsDNA) circles, and it is surprising that it encodes almost no viral structural proteins, although it harbors immunosuppressive genes that are expressed in the host and are essential for successful parasitism (4, 5) (see PDV description at www.ictvonline.org). Because of this lack of genes coding for structural proteins, it has been debated whether PDVs are of viral origin or a “genetic secretion” of the wasp (6, 7).

PDVs are classified as either bracoviruses or ichnoviruses, when associated with braconid or

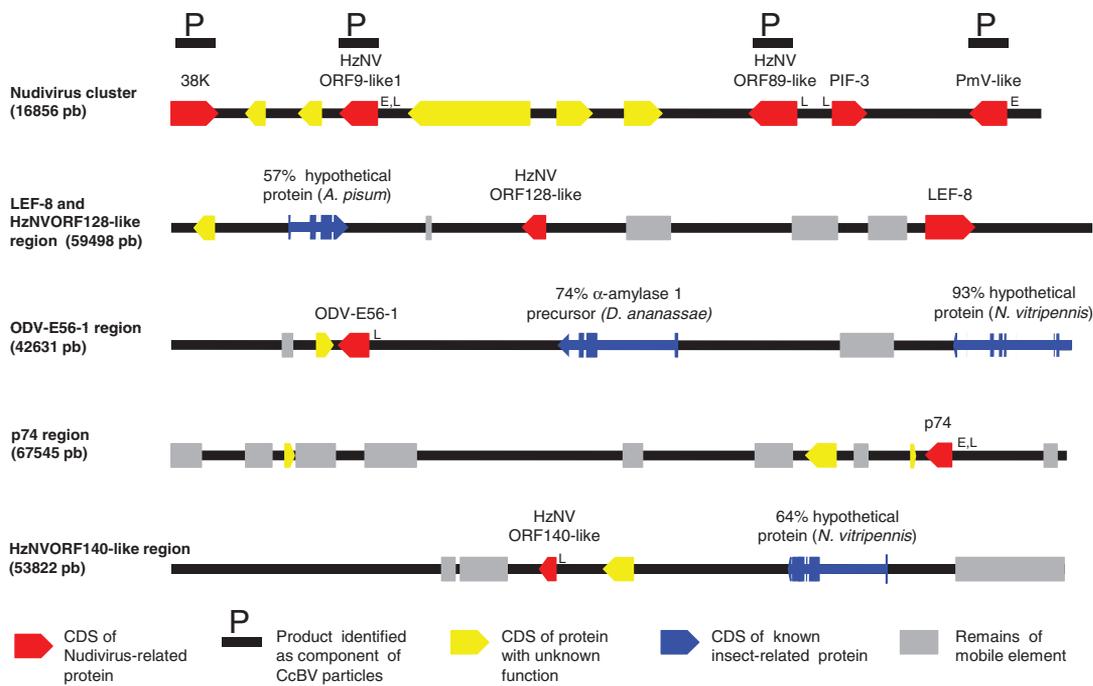
ichneumonid wasps, respectively. Detailed phylogenetic studies have shown that the bracovirus-associated wasps form a monophyletic group known as the microgastroid complex (8), and it has been hypothesized that there has been a single integration event of a viral genome, as a provirus, in the microgastroid lineage. This predicts that vertically transmitted viral DNA may have been maintained because of its contribution to successful parasitism and that PDVs have contributed to the diversification of the microgastroid complex of at least 17,500 species (8).

The sequence of the DNA packaged in *Cotesia congregata* bracovirus (CcBV) comprises 560 kb, organized in 30 circles of dsDNA (4), and encodes several products functionally resembling virulence factors used by parasites or bacterial pathogens of vertebrates (9–11). However, the origin of the protein components of PDV particles remains unknown. The few CcBV sequences with significant similarity to

¹Institut de Recherche sur la Biologie de l’Insecte, CNRS UMR 6035, Université François Rabelais, Parc de Grandmont, 37200 Tours, France. ²Institute of Cell Biology, University of Bern, Baltzerstrasse 4, CH-3012 Bern, Switzerland. ³Department of Clinical Research, Murtenstrasse 35, CH-3010 Bern, University of Bern, Switzerland. ⁴Commissariat à l’Énergie Atomique (CEA), Genoscope (Centre National de Séquençage), 2 rue Gaston Crémieux, CP 5706, 91057 Evry Cedex, France. ⁵Institut Fédératif de Recherche J. Roche (IFR11), Inserm, Centre d’Analyse Protéomique de Marseille, Faculté de Médecine, 51 boulevard Pierre Dramard, 13916 Marseille Cedex 20, France. ⁶Biologie Intégrative et Virologie des Insectes, INRA UMR 1231, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France.

*To whom correspondence should be addressed. E-mail: drezen@univ-tours.fr

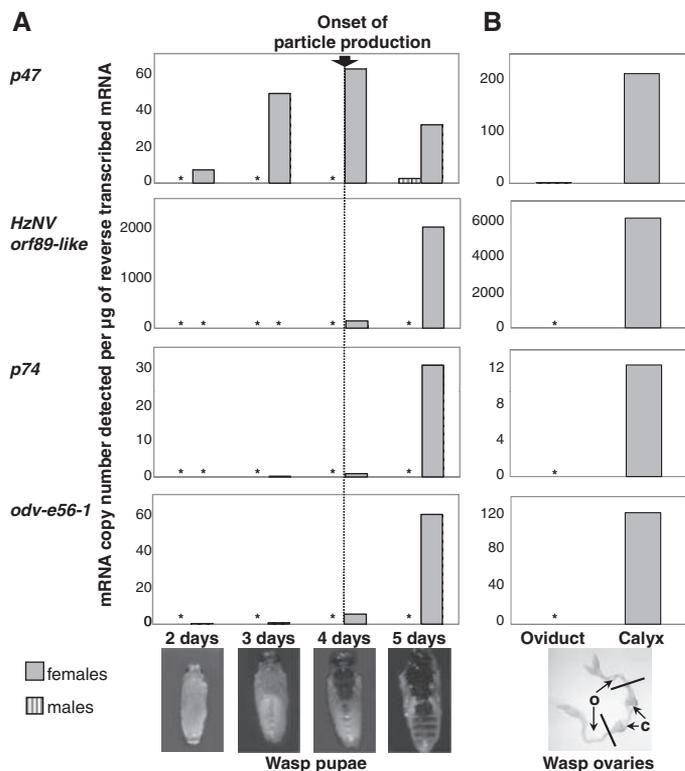
Fig. 1. Organization of nudivirus-related genes in the wasp genome (*Cotesia congregata*). Five genes (*38K*, *HzNVorf9-like1*, *HzNVorf89-like*, *pif-3*, and *PmV-like*) are organized as a cluster that is likely to constitute a remnant of the virus integrated into the ancestral wasp genome. Two genes (*HzNVorf128-like* and *lef-8*) are located within the same 60-kb chromosomal region; other genes (*odv-e56-1*, *p74*, and *HzNVorf140-like*) are dispersed and located in regions containing *Cotesia congregata* putative homologs of insect genes (in blue) and/or remnants of mobile elements (in gray). For nudivirus-related genes, the presence of sequences found in promoters of baculovirus genes transcribed by the cellular RNA polymerase (E) or baculovirus RNA polymerase (L) is indicated (19). E indicates the presence of a TATAA sequence with a CA(T/G)T or a CGTGC transcription start site 20 to 40 nucleotides downstream. L indicates the presence of a (A/T/G)TAAG motif within 300 nucleotides upstream of the translation start codon. CDS, coding sequence; ORF, open reading frame. *Acyrtosiphon pisum* (pea aphid), *Drosophila ananassae* (fruit fly), and *Nasonia vitripennis* (parasitoid wasp).



viral genes correspond mostly to remnants of mobile elements inserted randomly into the chromosomal form of CcBV and are thus not informative about the origin of bracoviruses (4). A viral genome without genes involved in particle production seems paradoxical. However, it may be that the genes involved in particle morphogenesis have lost the ability to be incorporated into the particles injected into the host, because the particles are exclusively produced in wasp ovaries and PDVs are only transmitted in their integrated chromosomal form. In accordance with this theory, a gene encoding an ichnovirus structural protein was identified in the wasp genome (12).

Regardless of where the genes are located, structural proteins of the particles are expressed in virus-producing cells. We therefore searched for virus-related genes expressed in wasp pupal ovaries at times when virus particle production is highest. We studied the braconid wasps *Chelonus inanitus* (Cheloniinae) and *Cotesia congregata* (Microgastriinae) belonging to different bracovirus-associated subfamilies (13). We also sequenced cDNAs from the wasp *Hyposoter didymator*, associated with an ichnovirus but belonging to the same superfamily (Ichneumonidea). We sequenced 5000 expressed sequence tags (ESTs) from the ovaries of each species and identified a set of nudivirus-related genes expressed by the braconid wasp ovaries (Table 1, A and B). The predicted products of these genes are related to 22 nudivirus genes (e values from $1e^{-65}$ to 1.1, see Table 1), 13 of which are conserved between nudiviruses and baculoviruses. In ichneumonid wasp ovaries, we detected mRNAs coding for characterized ichnovirus proteins but no genes

Fig. 2. Nudivirus-related gene expression correlates temporally with bracovirus particle production and occurs in the same tissue. (A) Real-time RT-PCR indicates that three nudivirus-related genes (*HzNVorf89-like* coding for a structural component of CcBV particles, *p74*, and *odv-e56-1*) are induced in female pupae from day 4, coincident with the initiation of particle production as detected by transmission electron microscopy (TEM) and PCR (26). The induction of *p47*, coding for one of the viral RNA polymerase subunits, occurs earlier, which would be expected if the viral RNA polymerase controls the expression of some nudivirus-related genes. Although baculovirus regulatory sequences are present in some promoters (see Fig. 1), they appear to be too short and, therefore, would lack the specificity necessary to selectively express chromosomally integrated genes. The four nudivirus-related genes are specifically expressed in the calyx region of the ovaries where bracovirus particles are produced, but not in the oviducts (B) nor in males (A). Asterisk indicates expression too low to be indicated. On wasp ovaries: o, oviduct; c, calyx.



related to known viruses. This suggests that the viral machinery of ichnovirus production may differ from that of bracoviruses. The recent discovery

of a possible new lineage of insect viruses, represented by the *Glossina pallidipes* salivary gland hypertrophy virus (14), illustrates that the diver-

sity of insect viruses is not completely known. This could explain our inability to identify viral genes in ichneumonid sequences. Few nudivirus genomes have been sequenced, and little is known about the function of their proteins, except by inference from studies on baculoviruses. Nudi-

viruses share half of the core set of essential genes conserved among baculoviruses (15, 16). The nudivirus-related gene products we identified in the braconid ovaries have been associated with different functions in baculoviruses (17–20) (Table 1) including subunits of the viral RNA polymerase

(LEF-4, LEF-8, and p47), proteins involved in particle assembly and packaging [38K (Ac98), VLF-1, and VP91], and envelope proteins of particles released after the death of infected insects (p74, PIF-1, PIF-2, and ODV-E56). In addition, we characterized several genes coding for variants

Table 1. Nudivirus-related genes expressed in braconid wasp ovaries and the presence of their products in baculovirus particles. Genes characterized in both *Cotesia congregata* (A) and *Chelonus inanitus* (B) are shaded in gray. Gene products identified as components of purified CcBV and CiBV particles by proteomic analyses are shown in red. HzNV-1, *Heliothis zea* nudivirus-1; GbNV,

Gryllus bimaculatus nudivirus; OrNV, *Oryctes rhinoceros* nudivirus; PmV, *Panaeus monodon* virus (shrimp nudivirus); AdorNPV, *Adoxophyes orana* nucleopolyhedrovirus. Dash indicates a transcript not found among the cDNAs sequenced. “No hit” indicates a protein having no blast hit but similar to a nudivirus-related protein of the other species. See table S1 for more details.

Gene type and function	Genes expressed in wasp ovaries producing bracovirus particles	Best blast		<i>C. congregata</i> protein			
		Nudivirus/baculovirus	Acc. no.	Name	Acc. no.	e value	Similarity (%)
Nudivirus/baculovirus core genes							
Transcription of viral genes	<i>RNA polymerase subunit lef-4</i>	HzNV-1	22788805	–	–	–	–
	<i>RNA polymerase subunit lef-8</i>	HzNV-1	22788797	CcLEF-8	CAR31572.1	1e ⁻⁶⁰	51
	<i>RNA polymerase subunit p47</i>	HzNV-1	22788782	Ccp47	CAR31573.1	2e ⁻⁰⁹	43
	<i>Transcript. initiation factor lef-5</i>	AdorNPV	209978847	CcLEF-5*	CAT00573.1	0.008	54
Packaging and assembly	<i>38K (Ac98)</i>	HzNV-1	22788860	Cc38K	CAR31574.1	1e ⁻²¹	57
	<i>Very late factor-1 Vlf-1</i>	PmV	160432005	–	–	–	–
	<i>vp91</i>	GbNV	134303400	–	–	–	–
ODV envelope components	<i>Per os infectivity factor p74</i>	HzNV-1	22788862	Ccp74	CAR31575.1	2e ⁻⁵⁴	47
	<i>Per os infectivity factor pif-1</i>	HzNV-1	22788762	–	–	–	–
	<i>Per os infectivity factor pif-2</i>	HzNV-1	22788829	–	–	–	–
	<i>Per os infectivity factor pif-3</i>	HzNV-1	22788795	CcPIF-3	CAR31576.1	3e ⁻¹⁵	51
	<i>odv-e56</i>	HzNV-1	22788783	CcODV-E56-1	CAR31577.1	3e ⁻¹²	41
Unknown	<i>19 kDa (Ac96)</i>	GbNV	134303485	CcODV-E56-2	CAR31578.1	8e ⁻²⁸	48
				Cc19kDa	CAR31579.1	7e ⁻¹⁰	51
Nudivirus/lepidopteran baculovirus core gene							
ODV envelope component	<i>odv-e66</i>	OrNV	108515114	CcODV-E66-1	CAR31580.1	No hit	–
				CcODV-E66-2	CAR31581.1	No hit	–
Nudivirus-specific genes							
Unknown	<i>HzNVorf9</i>	HzNV-1	22788719	CcHzNVORF9-like1	CAR31582.1	0.094	41
				CcHzNVORF9-like2	CAR31583.1	0.001	42
	<i>HzNVorf64</i>	HzNV-1	22788771	CcHzNVORF64-like	CAR31584.1	1e ⁻¹⁵	45
	<i>HzNVorf89</i>	HzNV-1	22788796	CcHzNVORF89-like	CAR31585.1	No hit	–
	<i>HzNVorf106</i>	HzNV-1	22788813	CcHzNVORF106-like	CAR31586.1	No hit	–
	<i>HzNVorf128</i>	HzNV-1	22788834	CcHzNVORF128-like	CAR31587.1	1e ⁻⁰⁵	43
	<i>HzNVorf140</i>	HzNV-1	22788845	CcHzNVORF140-like	CAR31588.1	8e ⁻⁰⁹	45
	<i>HzNVorf144</i>	PmV	160432004	–	–	–	–
	<i>PmV hypothetical protein</i>	PmV	134260388	CcPmV-like	CAR31589.1	0.003	44

Gene type and function	Genes expressed in wasp ovaries producing bracovirus particles	<i>C. inanitus</i> protein				Cc/Ci protein similarity (%)
		Name	Acc. no.	e value	Similarity (%)	
Nudivirus/baculovirus core genes						
Transcription of viral genes	<i>RNA polymerase subunit lef-4</i>	CiLEF-4	CAR40187.1	3e ⁻⁰⁴	39	–
	<i>RNA polymerase subunit lef-8</i>	–	–	–	–	–
	<i>RNA polymerase subunit p47</i>	–	–	–	–	–
	<i>Transcript. initiation factor lef-5</i>	–	–	–	–	–
Packaging and assembly	<i>38K (Ac98)</i>	Ci38K	CAR40188.1	6e ⁻¹⁸	58	66
	<i>Very late factor-1 Vlf-1</i>	CiVLF-1	CAR40190.1	2e ⁻⁰⁸	50	–
	<i>vp91</i>	CiVP91	CAR40191.1	8e ⁻⁰⁴	38	–
ODV envelope components	<i>Per os infectivity factor p74</i>	Cip74	CAR40192.1	5e ⁻⁶⁵	46	51
	<i>Per os infectivity factor pif-1</i>	CiPIF-1	CAR40193.1	3e ⁻³¹	45	–
	<i>Per os infectivity factor pif-2</i>	CiPIF-2	CAR40194.1	1e ⁻³⁹	48	–
	<i>Per os infectivity factor pif-3</i>	–	–	–	–	–
	<i>odv-e56</i>	CiODV-E56	CAR40195.1	7e ⁻⁰⁵	39	44
Unknown	<i>19 kDa (Ac96)</i>	Ci19kDa	CAR40196.1	2e ⁻¹²	53	41
						55
Nudivirus/lepidopteran baculovirus core gene						
ODV envelope component	<i>odv-e66</i>	CiODV-E66	CAR40197.1	8e ⁻¹²	41	54
						42
Nudivirus-specific genes						
Unknown	<i>HzNVorf9</i>	CiHzNVORF9-like1	CAR40198.1	0.009	41	82
		CiHzNVORF9-like2	CAR40199.1	No hit	–	74
	<i>HzNVorf64</i>	–	–	–	–	–
	<i>HzNVorf89</i>	CiHzNVORF89-like	CAR40200.1	0.094	40	60
	<i>HzNVorf106</i>	CiHzNVORF106-like	CAR40201.1	1.1	46	69
	<i>HzNVorf128</i>	CiHzNVORF128-like	CAR40202.1	1e ⁻⁰⁴	48	81
	<i>HzNVorf140</i>	CiHzNVORF140-like	CAR40203.1	9e ⁻¹⁰	48	56
	<i>HzNVorf144</i>	CiHzNVORF144-like	CAR40240.1	6e ⁻²⁰	51	–
	<i>PmV hypothetical protein</i>	CiPmV-like	CAR40204.1	0.064	42	69

*CcLEF-5 is slightly closer to AdorNPV than to HzNV-1 LEF-5.

of ODV-E66, a conserved envelope protein encoded by lepidopteran baculoviruses and a nudivirus (Table 1). We found no viral genes involved in DNA replication, which suggests that these mRNAs are rare or that host genes are involved.

The nudivirus *Heliothis zea* nudivirus-1 (HzNV-1) was originally identified as a persistent viral infection in a cell line from adult ovarian tissue of the moth *Heliothis zea* (21) and is closely related to HzNV-2, a sexually transmitted virus replicating in insect reproductive tissues (22). The genomes of HzNV-1; of *Gryllus bimaculatus* nudivirus (GbNV), a virus infecting the fat body of crickets; and of *Oryctes rhinoceros* nudivirus (OrNV), a biological control agent used to protect palm trees against the rhinoceros beetle have been sequenced (15, 23, 24). Partial data are also available for a shrimp nudivirus, designated here as PmV (GenBank accession number: 160432003). In braconid wasp ovaries, we identified eight ESTs similar to nudivirus-specific genes; two are shared between several nudiviruses (*HZNvorf64-like* and *HZNvorf144-like*); five have been found only in HzNV-1 (*HZNvorf9-like*, *HZNvorf89-like*, *HZNvorf106-like*, *HZNvorf128-like*, and *HZNvorf140-like*); and one is specific to the shrimp nudivirus (*PmV-like*). All braconid viral products have less than 60% similarity with nudiviral proteins (Table 1, A and B, e values $6e^{-20}$ to 1.1), which is not surprising, considering that the ancestral nudivirus is hypothesized to have integrated into the wasp genome 100 million years ago (25).

The prevalence of nudiviruses in hymenoptera is unknown, but the fact that nudivirus transcripts were not detected in the ichneumonid species indicates that parasitic wasps are not universally infected. The isolation of the nudivirus-related *p74* genes from eight *Cotesia* species using wasps collected in different parts of the world (fig. S1) indicated that this gene is stably associated with these wasps and does not belong to a pathogenic virus present in our laboratory strain. We could also show by sequencing *Cotesia congregata* large genomic DNA regions that at least 10 nudivirus-related genes are chromosomally integrated. Five genes were found to be clustered (*38K*, *HZNvorf9-like1*, *HZNvorf89-like*, *pif-3*, and *PmV-like*); others were dispersed (*HZNvorf128-like*, *lef-8*, *odv-e56-1*, *p74*, and *HZNvorf140-like*) and flanked by wasp genomic DNA (Fig. 1). Quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) showed that the expression of nudivirus-related genes increases in female wasp pupae (Fig. 2), coinciding with the onset of bracovirus replication (26). Moreover, expression in the ovaries is

confined to the calyx region where particles are produced (Fig. 2).

To confirm that the nudivirus-related genes produce bracoviruses, we analyzed proteins from purified particles by tandem mass spectrometry (MS/MS). We identified the products of six genes as components of the particles in both CcBV and CiBV (*Chelonus inanitus* bracovirus); the protein ODV-E66 in CcBV particles; and the proteins ODV-E56, p74, PIF-1, and PIF-2 in CiBV (indicated in red in Table 1, see table S2 for detailed results). Altogether, 20 different nudivirus-related gene products were identified as components of bracovirus particles, which demonstrated a functional link between the nudiviral machinery and bracoviruses.

Chelonus inanitus and *Cotesia congregata* belong to the most distantly related subfamilies of bracovirus-associated wasps (25), which suggests that the nudiviral machinery is present in all these species. Accordingly, we amplified the most conserved nudivirus-related genes (*HZNvorf9-like1* and *HZNvorf128-like*) from most wasp subfamilies of the microgastroid complex (Fig. 3). We conclude that these genes were present before the radiation of the complex and originated from a virus integrated in a chromosome of the ancestral wasp. The cluster

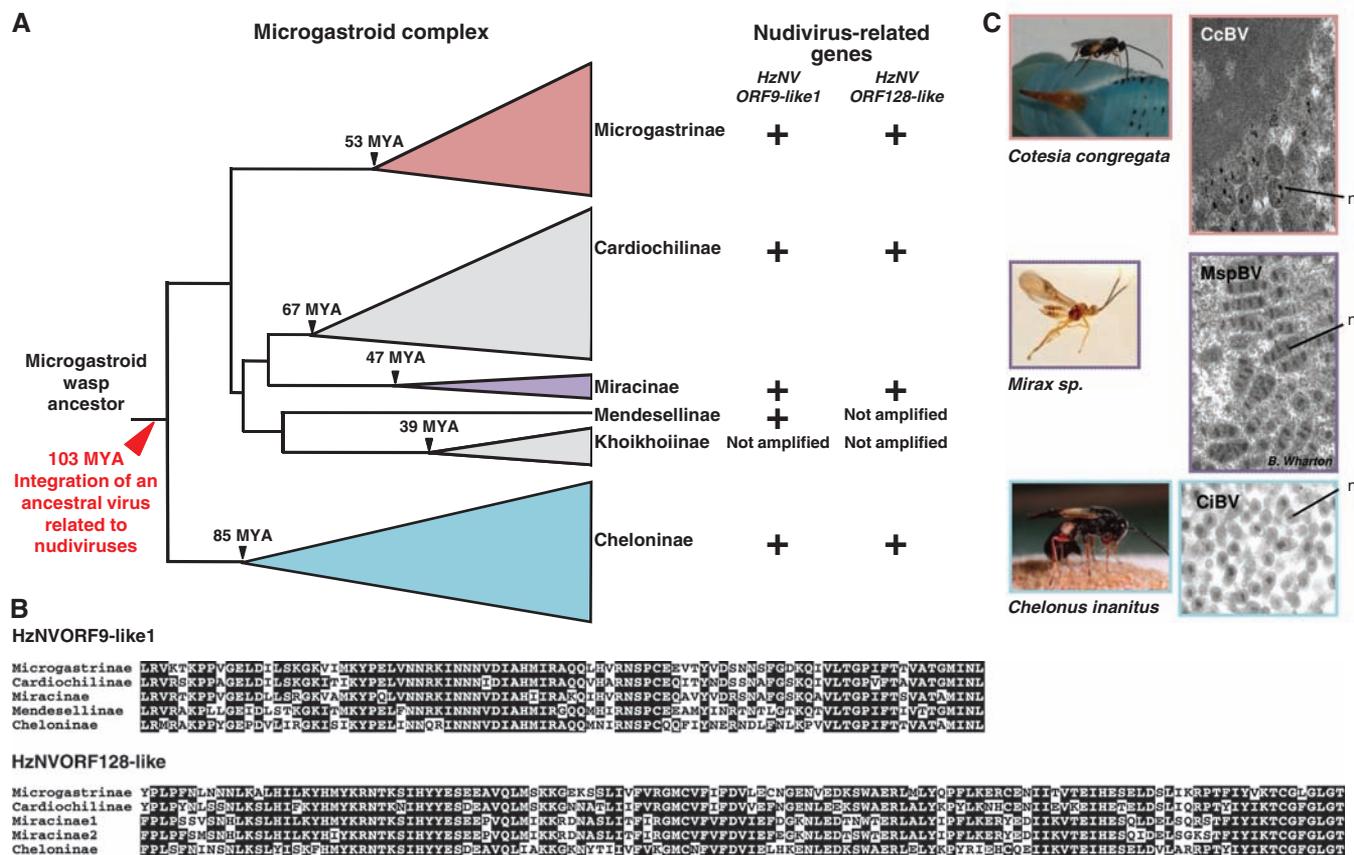


Fig. 3. Conservation of the machinery producing bracovirus particles among microgastroid wasps. The tree and dates of radiations are taken from (25). (A) Nudivirus-related genes amplified with DNA from species belonging to different subfamilies: *C. congregata* (Microgastrinae), *Toxoneuron nigriceps* (Cardiochilinae), *Mirax sp.* (Miracinae), *Epsilogaster sp.* (Mendesellinae), *Sania sp.*

(Khoikhoiinae), and *C. inanitus* (Cheloninae). (B) Alignment of HZNvorf9-like1 and HZNvorf128-like proteins deduced from the amplified sequences. (C) Bracovirus particles visualized by TEM from *C. congregata*, *Mirax sp.*, and *C. inanitus*. n, Nucleocapsid. The particles contain one (CiBV) or several nucleocapsids (CcBV and MspBV), dispersed (CcBV) or organized (MspBV).

of genes present in *Cotesia congregata* could constitute the remnants of the genome of this ancestral virus. The level of similarity between *HZNvorf9-like1* and *HZNvorf128-like* products from *Chelonus inanitus* and *Cotesia congregata* reaches 80% and thus indicates a strong conservation of their functions (Table 1). Some proteins show a lower conservation (for example for p74, 46% similarity), which suggests these sequences may be involved in more specific interactions with the host and have had to evolve more rapidly due to selective pressures associated with infection.

The overall conservation of the nudiviral machinery encoded by the wasps contrasts sharply with the lack of similarity found between the DNA enclosed in CiBV and CcBV particles. No common genes were found between CiBV and other bracovirus genomes (27); only sequences involved in the production of the circular dsDNAs of the particles (excision sequences) were conserved (28, 29). Furthermore, packaged bracovirus genomes do not contain any nudivirus-related genes. We hypothesize that shortly after initial integration of the nudivirus ancestor, viral DNA might have been replaced by wasp DNA in the particles (possibly by translocation of sequences allowing excision and encapsidation) and that most genes promoting parasitism were acquired later and independently in bracovirus-associated wasps.

It is well documented that genes of viral origin are used by eukaryotes to ensure physiological functions, such as the syncytins involved in trophoblast differentiation, which originated

from retroviral envelope proteins independently acquired by primates and mice (30). However, the bracovirus-wasp associations represent the only example, so far, of the incorporation of genes encoding a complex viral machinery that allows its eukaryotic host to transfer and express heterologous genes in target organisms. In this regard, unraveling bracovirus particle assembly could contribute to the design of new vectors for gene therapy.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5916/926/DC1

Material and Methods

Fig. S1

Tables S1 to S5

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Effects of Genetic Perturbation on Seasonal Life History Plasticity

Amity M. Wilczek,¹ Judith L. Roe,² Mary C. Knapp,² Martha D. Cooper,¹ Cristina Lopez-Gallego,^{1*} Laura J. Martin,^{1†} Christopher D. Muir,^{1‡} Sheina Sim,^{2§} Alexis Walker,¹ Jillian Anderson,¹ J. Franklin Egan,^{1||} Brook T. Moyers,^{1¶} Renee Petipas,^{1#} Antonis Giakountis,³ Erika Charbit,² George Coupland,³ Stephen M. Welch,² Johanna Schmitt^{1**}

Like many species, the model plant *Arabidopsis thaliana* exhibits multiple different life histories in natural environments. We grew mutants impaired in different signaling pathways in field experiments across the species' native European range in order to dissect the mechanisms underlying this variation. Unexpectedly, mutational loss at loci implicated in the cold requirement for flowering had little effect on life history except in late-summer cohorts. A genetically informed photothermal model of progression toward flowering explained most of the observed variation and predicted an abrupt transition from autumn flowering to spring flowering in late-summer germinants. Environmental signals control the timing of this transition, creating a critical window of acute sensitivity to genetic and climatic change that may be common for seasonally regulated life history traits.

The seasonal timing of critical life history events is often under strong natural selection, requiring plants to integrate and respond appropriately to multiple environmental signals. In the case of flowering time, these in-

clude day length, ambient temperature, and winter chilling (vernalization) cues. Genetic mechanisms of flowering response to environmental cues have been well characterized in the model plant *Arabidopsis thaliana* in the laboratory. However,

the response of *Arabidopsis* to complex, natural environmental cues is unknown. The native distribution of *Arabidopsis* encompasses climates from the Mediterranean, where it is a winter annual that germinates in late autumn and flowers in late winter or early spring, to northern Scandinavia, where the species germinates in early autumn, overwinters, and flowers in late spring and early summer. In England and northwestern Europe, *Arabidopsis* either is a winter annual or undergoes rapid cycling by germinating in early autumn, spring, or summer, thus flowering without vernalization (1, 2). Thus, in nature the species experiences marked variation in photothermal environments across sites and seasons.

The timing and environmental sensitivity of flowering in *Arabidopsis* are regulated by a network of genes in several converging pathways (3–5) (fig. S1). Inductive long days are perceived by the photoperiod pathway, which accelerates flowering acting through *GIGANTEA* (*GI*) and *CONSTANS* (*CO*) (6, 7). The gibberellin pathway (8) and high temperatures (9–12) also promote flowering. These signals activate floral integrator genes such as *FLOWERING LOCUS T* (*FT*) (3). The ability of these integrators to respond is controlled by repressor genes, notably *FLOWERING*