

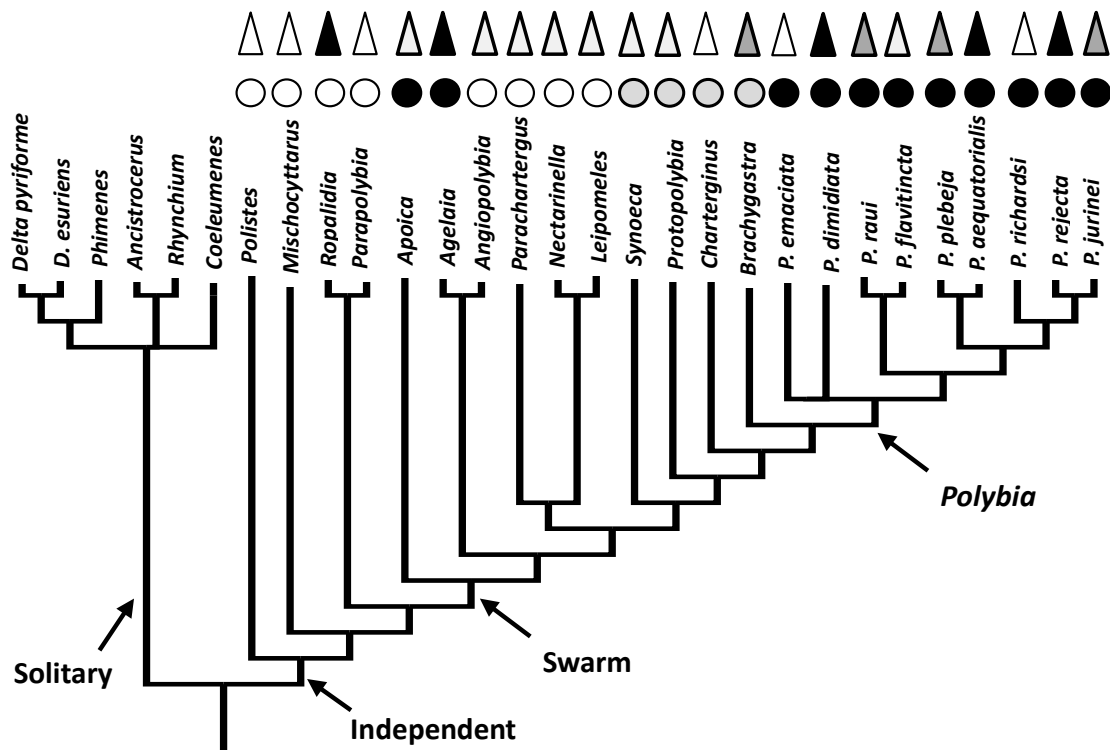
### Supplementary information.

**Subject species, collection dates and locations were:** *Polybia jurinei*: November 1994, Ecuador, 0°40.5'S, 76°25.8'W; *Polistes instabilis*: July 2005, Costa Rica, 10°27.2'N, 85°7.5'W; *Agelaia xanthopus*, *Mischocyttarus mastigophorus*, *Polybia emaciata*: August 2006, Costa Rica, 10°18.1'N, 84°47.9'W; *Nectarinella championi*, *Polybia raii*: August 2006, Costa Rica, 10°14.4'N, 84°54.3'W; *Apoica pallens*, *Angiopolybia zischkai*, *Charterginus fulvus*, *Leipomeles dorsata*, *Parachartergus smithii*, *Polybia dimidiata*, *Polybia richardsi*, *Protopolybia exigua*, *Synoeca septentrionalis*: June 2007, Ecuador, 0°40.3'S, 76°24.0'W; *Polybia flavitincta*: March 2012, Costa Rica: 10°25.6'N, 84°1.2'W *Brachygastra smithii*, *Polybia aequatorialis*, *Polybia plebeja*, *Polybia rejecta*: July 2012, Costa Rica 10°16.3'N, 84°49.4'W; *Ancistrocerus* sp., *Coeleumenes burmanicus*, *Delta esuriens*, *Delta pyriforme*, *Parapolybia varia*, *Phimenes flavopictus*, *Rhynchium quinquecinctum*, *Ropalidia fasciata*: May 2014, Taiwan: 21°57.8'N, 120°49.5'E.

**Histology and neuroanatomy methods:** We cut the fixed wasps' head capsules from the thorax and dehydrated through a series of increasing ethanol concentrations, acetone, then through increasing concentrations of plastic resin (resin composition: 5.5 g of EMbed 812 (a mixture of bisphenolA/epichlorohydrin epoxy resin; (CAS #25068–38-6), epoxy modifier (CAS #2425–79-8), 5.7 g of dodecyl succinic anhydride, 0.65 g of dibutyl phthalate, and 0.31g of 2,4,6-tri(dimethylaminoethyl)phenol. We incubated individual wasp heads in 0.1 ml resin in pyramid molds at 60°C for 72 hours, then glued the resin to 0.5 ml acrylic cylinders with cyanoacrylate adhesive and cut each head along the frontal plane into 12 to 16 µm thick sections (depending on species) using a rotary microtome with disposable steel histology blades. Sections were mounted on gelatin-coated microscope slides and the tissue was stained with Toluidine blue. We cleared the stained sections in a series of increasing ethanol concentrations and cover slipped under transparent mounting medium.

We used a microscope-mounted digital camera to photograph the tissue sections at 2560 X 1920 pixel resolution, using 2.5 X or 5 X microscope objectives (depending on species). For each wasp, we

began photographing every other section at the section where brain tissue first became visible. ImageJ version 1.46 digital imaging analysis software (<http://rsbweb.nih.gov/ij/>) was used to quantify the volumes of brain structures. We outlined the target brain regions and quantified the number of image pixels in the structure using ImageJ, and then converted the pixel counts to area using a photograph of a stage micrometer taken at the same resolution and magnification as a size reference. We multiplied the areas by section thickness to yield volume.



**Supplemental figure.** Phylogeny of the 29 species sampled in our analysis, labeled by genus excepting where more than one species was sampled [30-32]. Labels at internal branches indicate modes of colony founding. For the social species, colony characteristics are indicated in the symbols above the taxon names. Circles indicate degree of caste differentiation: white: no detectable caste differences, gray: queens larger or physiological castes, black: morphologically distinct queens [41]. Triangles indicate colony size categories: white: 25-100, light gray: 101-200, dark gray: 200-500, black:>1,000.