Matricide, or the killing of the mother queen by workers, is a dramatic example of intracolony conflict in some species of annual yellowjacket wasps, but the selective pressures that have produced this behavior are poorly understood. Matricide may be very costly because queens cannot be replaced in annual colonies, and following queen death, no new females (workers or reproductives) can be produced. Here we provide the first description of matricide in *Dolichovespula arenaria* from three video-recorded observations. Further, we present the first direct tests of two hypotheses that could explain the intraspecific variation in occurrence of matricide. The male production hypothesis suggests that workers execute queens that are producing primarily (or exclusively) male brood, because workers are more related to worker-produced males than to queen-produced males. An alternative explanation for matricide, the unrelated-worker hypothesis, suggests that workers are unrelated to the queen they kill because they are the daughters of a usurped queen or they are socially parasitic drifters from a foreign colony. We tested the male production hypothesis by experimentally creating male-laying queens via surgical removal of the queen’s spermatheca in colonies entering the reproductive stage. Colonies were then observed to determine if male production induces matricide, compared to controls without spermatheca removal. To test the unrelated-workers hypothesis, we looked for a correlation between multiple matrilines and observed or inferred matricides. Our results suggest that queen-killing workers are daughters of the queen but do not respond to queen sex investment, counter to theoretical predictions. We suggest that manipulating queen signals may be a promising next approach to determining the proximate and ultimate triggers for matricide.