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Disentangling Nature versus Nurture in a Mouse Model of Alcohol Use

Laura Bauer
SUNY Geneseo

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Abstract

Alcohol use disorder (AUD) is a major societal concern and so understanding how it develops is an important topic of research. Epigenetic mechanisms induced by mother-infant interactions can turn genes on or off in response to being reared in a different environment. This research attempted to disentangle the roles of nature and nurture on the development of AUD. Two strains of inbred mice were cross-fostered to see if the postnatal rearing environment can reduce alcohol consumption in the strain of mice genetically predisposed to drink alcohol. Researchers recorded mouse maternal care, pup anxiety, and pup alcohol consumption. It was hypothesized that mice that are predisposed to drink alcohol (B6 strain) will drink less when reared by mothers not predisposed to drink (FVB strain), potentially via maternal care differences. It was also hypothesized that mice that are not predisposed to drink alcohol (FVB strain) will drink more when reared by mothers predisposed to drink (B6 strain). These data tested the ability of the early maternal environment to shift the behavior of offspring genetically predisposed to drink alcohol. Results showed that FVB mice and cross-fostered mice were less anxious overall, and that female mice, B6 mice, and non-fostered mice tended to drink the most of any group. These findings imply that early life trauma alongside epigenetics can influence anxiety and alcoholic tendencies later in life.

The lifetime prevalence of alcohol use disorder (AUD) in the United States is 29.1% (Grant et al., 2015). Thus, understanding the complex etiology of AUD has the potential to benefit those suffering from AUD. Disentangling the role of genetic, epigenetic, and environmental influences on behavioral outcomes can be studied using animal models.

Inbred strains of mice have relatively fixed genetic sequences, so phenotypic differences between strains of mice are often ascribed to differences in genetic background. It is known that B6 mice will voluntarily drink alcohol (10% EtOH unsweetened) in much greater amounts than other strains. In a comparison of 22 inbred strains, B6 mice drank

the most, and five times more than FVB mice (Yoneyama et al., 2008). This study also found that the proportion of the variance in consumption of unsweetened alcohol that could be explained by genotype was 44–60% (Yoneyama et al., 2008). It has also been found that female B6 mice tend to consume more alcohol than their male counterparts (Middaugh et al., 1999). However, several impactful animal studies investigating the effects of early environment on behavior suggest mother-infant interactions lead to long-term developmental changes via epigenetic changes (Liu et al., 1997; Weaver et al., 2004).

This research will investigate the interaction between genotype and environment on the development of alcohol use in early adulthood. One strategy used in rodents to investigate the effects of rearing environment on strain differences in behavior is postnatal cross-fostering. The mouse strains used are two well-characterized inbred mouse strains: C57BL6/J (B6) and FVB/NJ (FVB) to investigate this interaction by examining the influence of postnatal cross-fostering on the behavioral development of offspring. Thus, researchers aim to explore the interaction between pup experience (fostered B6, fostered FVB, or non-fostered) and maternal strain (B6, FVB) on offspring alcohol use during early adulthood. This research will assess maternal care as one potential mediating mechanism. As previous data suggest females may suffer more from the shifts in early environment that reduce maternal care (e.g., Bechard et al. 2012). Sex-dependent effects of fostering on alcohol use will also be explored.

This research is composed of two hypotheses. The first hypothesis claims that B6 (i.e., genetically predisposed to prefer alcohol) mice reared by FVB dams will consume less alcohol during early adulthood than B6 mice raised by their biological mothers. The second hypothesis claims that FVB mice (i.e., that are not predisposed to drink alcohol) will show a slight increase in alcohol consumption when reared with B6 mothers compared to FVB mice reared by their biological mothers.

Methods

Cross-Fostering

Cross-fostering is when mouse pups are weaned from their biological mothers at birth and swapped with similarly aged pups from a different dam, so the pups are reared by the opposite dam. Parent mice were set up in groups comprised of one male and two females of the same strain (i.e., 8 dams/strain). Mating was timed to produce litters within 3 days of each other. Matings that did not result in coinciding births were assigned to the control (non-fostered) group where they were reared with their biological dam. Whenever possible, coinciding whole litters were swapped between a B6 and FVB dam until 12 pups (about 2–3 litters) of each strain were cross-fostered.

Offspring subject groups:

1. B6 pups reared with B6 mothers (n=18)
2. FVB pups reared with FVB mothers (n=19)

3. B6 pups reared with FVB mothers (n=14)
4. FVB pups reared with B6 mothers (n=17)

Table 1: Ethnogram for specific behviors and their definitions

Behavior	*Definition*
Resting with pups (RP)	Mother is resting in contact with pups including nursing (solely tail contact not included).
Active (A)	Mother is moving around, nesting, eating, or drinking and not in close contact with pups.
Inactive (IA)	Mother is not moving around and is still for at least three seconds and not in contact with pups.
Licking/grooming pups (LGP)	Mother is licking or grooming a pup.
Licking/grooming self (LGS)	Mother is licking or grooming herself.

Maternal Observations

Once the pups were cross-fostered, maternal care was observed for the next seven days per dam and observations were recorded for days one, three, and five. An ethogram for specific behaviors was generated (see *Table 1*). Maternal observations were carried out through live scoring in which a researcher was physically present and recorded specific maternal behaviors. Maternal observations were carried out for an hour each day until pups were seven days old. Within the hour, one mouse was observed for each minute as the researcher recorded the first behavior they saw for that designated minute. This led to 12 behaviors for each dam in an hour and this data was recorded for maternal observations. It is important to note that behaviors recorded from the ethogram are mutually exclusive.

Light Dark Box

Once pups reached 42 days, they were then tested in a Light Dark Box (LDB) as a measurement of general anxiety. Mice who explore the light side of the chamber are typically seen as less anxious than mice that spend more time in the dark side of the chamber (Bourin & Hascoet, 2003). Each mouse was placed in a chamber for 10 minutes where one side of the chamber is uncovered and flooded with light, while the other side is covered with dark paneling. To start the trials, the mouse is placed in the center of the chamber and this initiates an automatic recording of the mouse's path sequence through a computer processing system connected to the LDB, and they are free to roam. The chamber is cleaned between each session to ensure that there are no olfactory confounds. The time spent in the light and dark side by each mouse was automatically recorded through the computer processing system. All subjects were handled prior to the start of the experiment and habituated to the environment for at least 30 minutes prior to any session to control for any anxiety and to ensure the accurate recording of baseline behavior.

Drinking in the Dark

Intermittent Drinking in the Dark (iDID) is an established mouse drinking paradigm (e.g., Rodriguez-Ortiga et al. 2018) and was used to quantify alcohol consumption for each mouse. During a 2-hour test session that occurs during the dark (i.e., lights off) cycle, all mice were removed from their standard environment and placed in separate cages with access to 10% alcohol. The alcohol is delivered via 10 mL modified pipettes so that pre-session and post-session volumes can be used to calculate the individual alcohol consumption for each mouse per day, and escalation of use over time. Mice were tested in five sessions/week for 20 sessions in total. Sessions were held Monday, Tuesday, Thursday, and Friday from 9–11 and 12–2. Both B6 and FVB mice were equally balanced in sessions for both control and fostered sessions; this was to ensure that time of the sessions was not a confound in the results. All subjects were handled prior to the start of the experiment and habituated to the drinking environment for at least 30 minutes prior to any session to control for any anxiety and to record accurate baseline behavior.

The hypotheses for this research are as follows: B6 mice reared with FVB mothers will show a decrease in alcohol consumption compared to their non-fostered conspecifics (B6 reared by B6). FVB mice reared with B6 mothers may show a slight increase in alcohol consumption compared to their non-fostered conspecific (FVB reared by FVB). It was also predicted that B6 mice reared with B6 mothers would drink alcohol, while FVB mice reared with FVB mothers would not.

Results

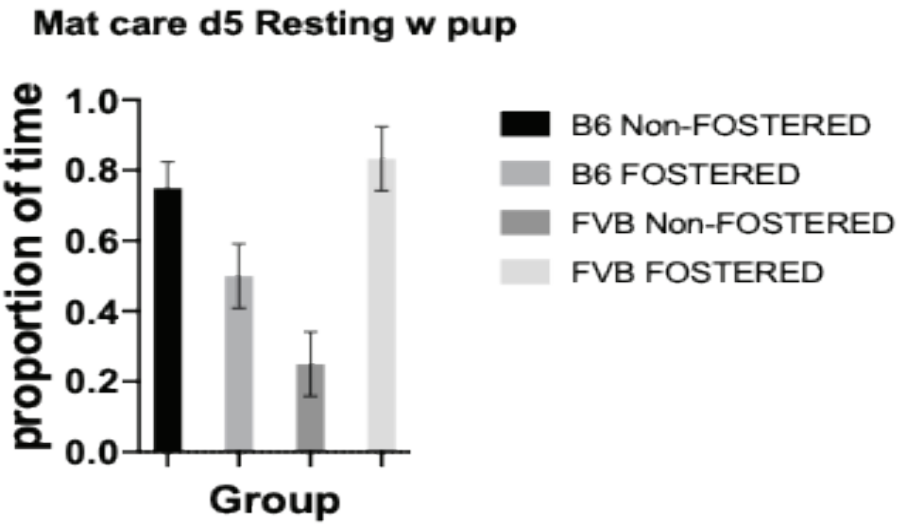

Figure 1: Maternal Care Day 5 Licking/Grooming Pups. Shows the amount of time dams were licking and grooming their pups across non-fostered groups on Day 5. FVB dams exhibited more licking and grooming behavior towards their pups slightly more than B6 dams ($F(1, 5) = 9.16$, $p = 0.029$). There were no differences in the amount of time dams spent self-grooming ($p>0.05$).

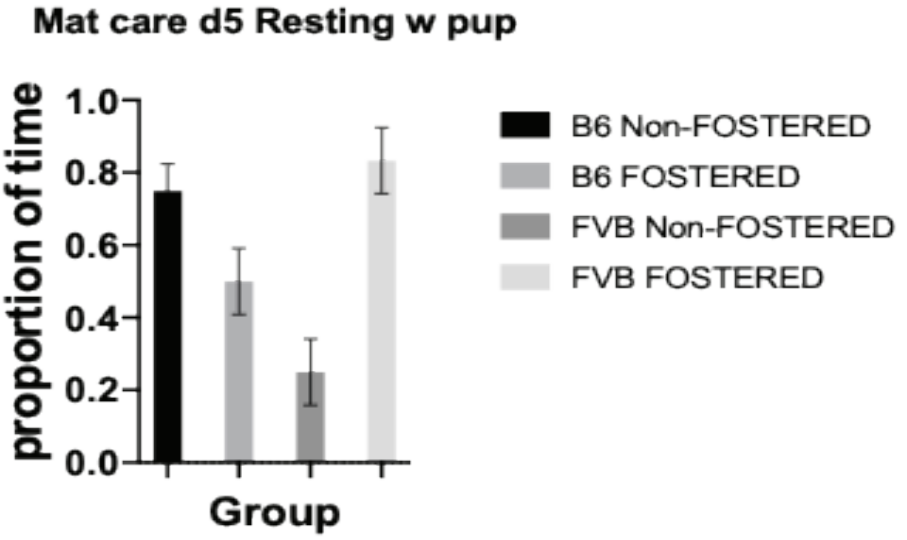

Figure 2: Maternal Care Day 5 Resting with Pups. Shows the amount of time dams were resting with pups across non-fostered and fostered groups on Day 5. The amount of time spent resting with the pups resulted in a strain by group interaction ($F(1, 5) = 22.72$, $p = 0.005$).

Maternal Observations

Unfortunately, due to COVID-19 restrictions, maternal observations were not able to be completed consistently. In one group, we were able to perform live observations of maternal care. With Covid restrictions, we did not complete maternal observations of non-fostered mice on days 1–4. Moreover, for the fostered mice we were forced to video record the maternal care observations made of this pre-recorded behavior. Thus, we present day 5 maternal care behavior only and acknowledge this confound prevents us from reliably interpreting the current data. This is a limitation to interpreting our findings, and we are currently aiming to repeat the maternal observations using simultaneous live and pre-recorded observations in a separate group of control mice to strengthen our interpretation of findings. Results on day 5 are as follows: dams with non-fostered litters were the only group to be observed spending time licking and grooming their pups in which no significant differences were observed (see *Figure 1*). The amount of time spent resting with the pups resulted in a strain by group interaction in which dams with B6 pups rested with them more than dams with FVB pups (see *Figure 2*). There were no differences in the amount of time dams spent self-grooming.

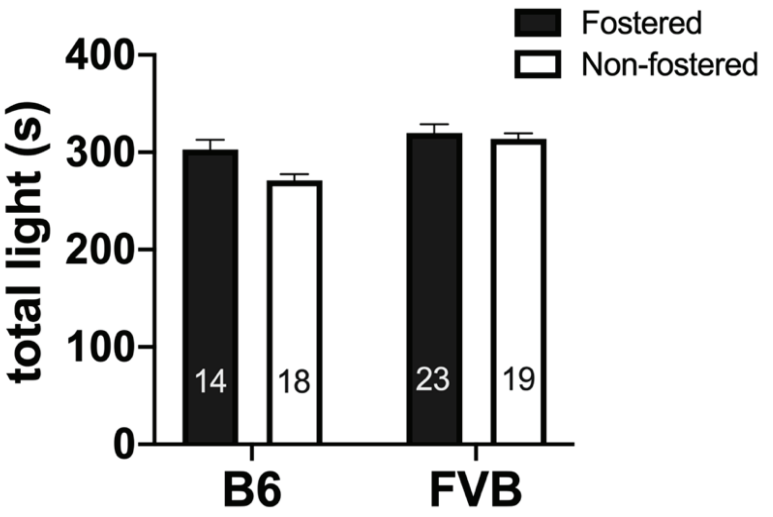

Figure 3: Total Time in the Light in Control and Fostered Mice. Shows the amount of time in the light side of the Light-Dark Box during a 10-minute test. Fostered mice spent more time in the light side than non-fostered mice (Fostered: $F(1, 97) = 5.75$, $p = 0.018$) and FVB mice spent more time in the light side than B6 mice (Strain: $F(1, 97) = 14.35$, $p < 0.0001$). No effect of Sex or interactions.

Light Dark Box

The LDB yielded interesting results. It was found that fostered mice spent more time in the light side of the chamber than non-fostered mice (see *Figure 3*). From this it can be deduced that fostered mice were less anxious and more exploratory than non-fostered mice. This is a significant finding as it implies that the act of cross-fostering mice in early life may reduce anxious tendencies later in life compared to mice reared with their own mothers. Another significant finding is that the FVB mice spent more time on the light side of the chamber than the B6 mice (see *Figure 3*). Similarly, the result concludes that FVB mice are less anxious than B6 mice. This data from the LDB yielded no effects of sex and also no interactions.

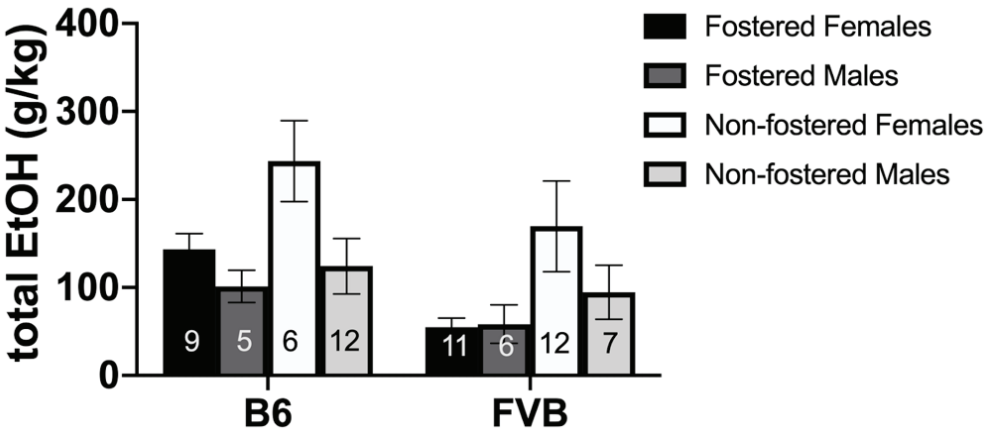

Figure 4: Total Alcohol Consumption in Control and Fostered Mice. Shows the total amount of EtOH (g/kg) consumed across sessions. B6 mice drank more than FVB mice (Strain: F(1, 60) = 4.92, p = 0.03). Non-Fostered mice drank more than fostered mice (Fostered: F(1, 60) = 6.71, p = 0.012). Females drank more than males (Sex: F(1, 60) = 4.84, p = 0.032). No interactions were significant.

Drinking in the Dark

There were multiple significant effects that resulted from the intermittent drinking in the dark. Firstly, it was found that B6 mice consumed more alcohol than FVB mice across control and experimental groups (see *Figure 4*). Secondly, there were sex effects as females drank more than males in both the B6 and FVB strains. Lastly, non-fostered mice consumed more alcohol than fostered mice; this was a profound finding as it is consistent with results from the LDB where cross-fostered mice may be less stressed and therefore less prone to drinking than control mice. This introduces the notion of anxiety and its role, in combination with epigenetics, on the effects of alcohol consumption. Overall, B6 and FVB non-fostered females consumed the most alcohol. These findings support prior research and confirm that female mice and B6 mice are prone to consume alcohol more than male and FVB mice. However, these findings also implicate that cross-fostering in early life may have an opposite effect on alcohol consumption, making non-fostered mice at higher risk to drink than fostered mice. There were no significant interactions found.

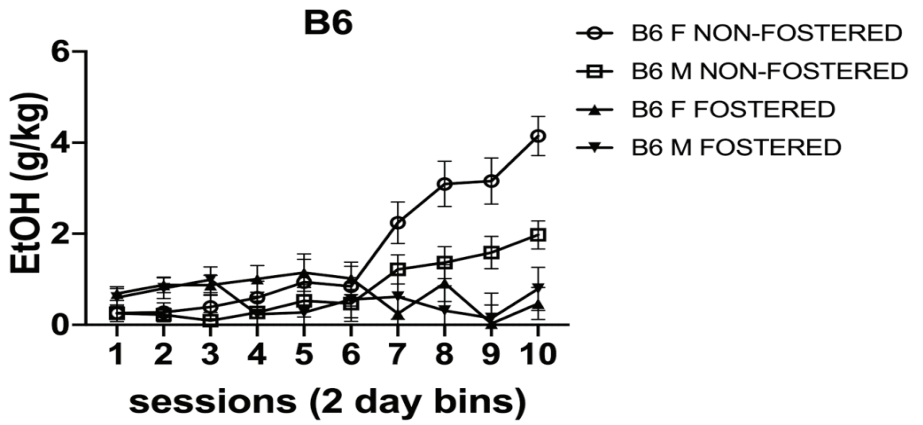

Figure 5: Alcohol Consumption Over Time in Control and Fostered B6 Mice. Shows the amount of alcohol consumed across sessions in female and male fostered and non-fostered B6 mice. Females consumed more alcohol than males, while non-fostered mice drank more than fostered mice.

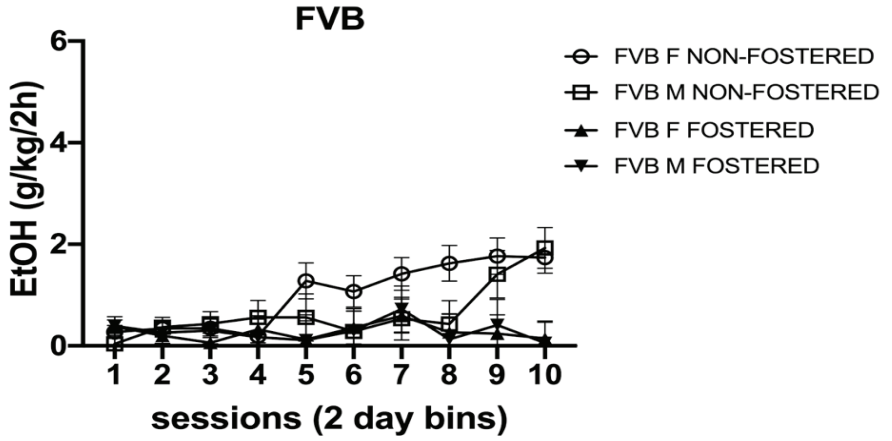

Figure 6: Alcohol Consumption Over Time in Control and Fostered FVB Mice. Shows the amount of alcohol consumed across sessions in female and male fostered and non-fostered FVB mice. Females consumed more alcohol than males, while non-fostered mice drank more than fostered mice.

Discussion

This research aimed to answer the question if rearing mice with different mothers who are either predisposed or not predisposed to drinking alcohol would have any effect on the pup's own predisposition to drink or not drink alcohol via maternal care and epigenetics. Of the two hypotheses that were established, only one was supported. The first hypothesis predicted that B6 pups predisposed to drink alcohol reared with FVB mothers would drink less than B6 pups reared with their own B6 mothers. This hypothesis was supported (see *Figure 5*). The second hypothesis predicted that FVB pups not

predisposed to drink alcohol reared with B6 mothers would drink slightly more alcohol than FVB pups reared with FVB mothers. However, this was not supported; the opposite occurred. FVB pups that were cross-fostered drank less than FVB pups who were not cross-fostered (see *Figure 6*). This raises many interesting questions.

This difference in alcohol consumption could possibly be from differences in mothering styles between FVB and B6 mothers. However, since maternal observations are not available at this time, no reliable conclusions can be made. Given the results of this study, another possible explanation may be that cross-fostering early in life may have served as a protective agent against anxiety and alcohol predisposition later in life. Future research should focus on examining the effects of early versus late trauma on anxiety and alcohol consumption in mice. It has been hypothesized that early trauma may actually act as a protective agent while late trauma may act as a risk factor for mice. This may explain why fostered mice were less anxious and drank less overall than non-fostered mice. Future research may want to examine if cross-fostering in early life will act as a protecting agent and lead to less anxiety and drinking, while cross-fostering later in life will act as a risk factor and lead to increased anxiety and drinking.

One limitation of this study is that maternal observations could not be completed. This is currently being corrected and data will hopefully be available soon. In order to prevent future errors, maternal observations should be live-scored and also recorded. With these two scores supplemented, researchers can ensure the accuracy of maternal observations and may also make observations remotely from watching recordings of the mice. Another limitation is that this research did not have an in-strain cross-fostering control comparison. This may lead to a confound in the results. Currently, strict conclusions cannot be made about the reason for these results and if these findings are due to differences in rearing or simply from the act of cross-fostering. There is a current project that is looking to rectify this. This future research is looking to cross-foster B6 pups with a different B6 mother as well as cross-fostering FVB pups with a different FVB mother and test these pups in the LDB and measure their alcohol consumption. This will then control for the act of cross-fostering on anxiety and drinking behaviors. By having a non-fostered group, a cross-fostered group between strains, and a cross-fostered group within strains, the act of cross-fostering on alcohol consumption will not be a confound.

Alcohol abuse is a serious issue, and these findings are significant to understand the underlying mechanisms behind it. As research continues, some potential causes and solutions may be discovered to better understand alcohol abuse and how it may be placated.

References

Bechard, A., Nicholson, A., & Mason, G. (2012). Litter size predicts adult stereotypic behavior in female laboratory mice. *Journal of the American Association for Laboratory Animal Science, 51*(4), 407–411.

Bourin, M., & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology*, *463*(1-3), 55–65. https://doi.org/10.1016/s0014-2999(03)01274-3

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P., Meaney, M. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*, *277*(5332), 1659–1662. https://doi.org/10.1126/science.277.5332.1659

Rodríguez-Ortega, E., & Cubero, I. (2018). Environmental enrichment modulates drug addiction and binge-like consumption of highly rewarding substances: A role for anxiety and compulsivity brain systems? *Frontiers in Behavioral Neuroscience*, *12*, Article 295. https://doi.org/10.3389/fnbeh.2018.00295

Weaver, I.C.G., Cervoni, N., Champagne, F., D'Alessio, A., Sharma, S., Seckl, J., Dymov, S., Szyf, M., & Meaney, M. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*(8), 847–854. https://doi.org/10.1038/nn1276

Yoneyama, N., Crabbe, J.C., Ford, M.M., Murillo, A., & Finn, D.A. (2008). Voluntary ethanol consumption in 22 inbred mouse strains. *Alcohol*, *42*(3), 149–160. https://doi.org/10.1016/j.alcohol.2007.12.006